EFFECT OF NUTMEG SEEDS EXTRACTS (MYRISTICA FRAGRANS HOUTT) ON STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCUS EPIDERMIDIS

Sri Agung Fitri Kusuma¹*, Ronato² and Ardian Baitariza²

¹Department of Biology Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia 45363.
²Departement of Pharmacy, Faculty of Mathematics and Natural Sciences, Al-Ghifari University, Bandung, West Java, Indonesia.

ABSTRACT

Objective: This study was designed to compare the antibacterial effect of nutmeg seed extracts (Myristica Fragrans Houtt) on the common causative bacteria of skin infection, Staphylococcus aureus and S. epidermidis. Methods: The antibacterial effect of the nutmeg seed extracts was analyzed using the agar diffusion method. The determination of extracts Minimum Inhibitory Concentration (MIC) was done using the macrodilution method. In addition to the antibacterial effect conclusion, the antibacterial effect of the extract was evaluated on both staphylococcus strains using the neomycin sulfate as the standard antibiotic. The comparative method was the agar diffusion method, followed by the inhibitory diameters data plotting to log concentration of the extract or neomycin sulfate, thus an inhibition curve was obtained. Results: The antibacterial activity of nutmeg seed extract on S. aureus was stronger then to S. epidermidis. This data was supported by comparing the MIC value of the extract on both strains. The MIC value of the extract against S. aureus (0.156<x≤3.125%w/v) was lower than to S. epidermidis (0.3125<x≤ 0.625%w/v). In comparison with neomycin sulphate, the comparative activity value of the test extract against neomycin sulfate on S. aureus was 1: 11.119, while on the S. epidermidis was 1: 15.865. Conclusion: The nutmeg seed extract is more potential to be a antibacterial agent for infections caused by S. aureus than S. epidermidis.

INTRODUCTION

*Staphylococcus* is a bacterial genus that commonly found on human skin.\(^1\) In addition, Staphylococci are the most important pathogens in polymer-associated infections and cause a wide range of infections associated with prosthetic joints and other implanted biomaterials.\(^2\)-\(^4\) These bacteria can cause minor skin infections or doesn’t cause infectious disease at all. However, serious infections can be occurred if Staphylococcus enters into the bloodstream and it may lead to sepsis or death. The type of infections depends on the strain of Staphylococcus.\(^1,5\) Among them, *Staphylococcus aureus* is a major human pathogen that causes a wide range of clinical infections.\(^5\) Humans are a natural reservoir of *S. aureus*, with 30 to 50% of healthy adults colonized, 10% to 20% persistently so.\(^6,7\) *S. aureus* also well known as nosocomial infections causative pathogen as at hospital.\(^8\) *S. aureus* is capable of biofilm formation among other Gram-positive bacteria.\(^9\) The biofilms formation has been studied as a potential contribution in the *S. aureus* pathogenesis. *S. aureus* is the important pathogen that responsible for several infections, such as skin infections, recurrent and chronic airway infections, mastitis, and osteomyelitis.\(^10\)-\(^13\) Besides *S. aureus*, *S. epidermidis* is also known as a commensal pathogen in humans. *Staphylococcus epidermidis* is normally found on human skin and reported as a bacterial forming biofilms when it expresses polysaccharide intracellular adhesin (PIA). Therefore, PIA Production is a one of its virulence factor that is associated opportunistic infections.\(^14\) *S. epidermidis* is reported to be responsible for 35% to 60% of infections of synthetic urinary sphincters and penile prostheses.\(^15\)

*Staphylococcus aureus* and *S. epidermidis* generally produce penicillinases enzymes which break the penicillin β-lactam ring and lead to the antibiotic resistant.\(^16\) Recently, the global problem of bacterial resistance development of synthetic antibiotics has led scientists to consider the use of antibiotic substances found in the nature e.g. medicinal plants.\(^17\) The herbal medicine is currently used in health system worldwide and it is increasing widespread in developed countries.\(^18\) Several medicinal plants were reported for their several therapeutic, such as antiinfectious, antioxidant, and anti-tumor activities. These therapeutic effects are due to their bioactive compounds content.\(^19,20\) Mostly, the herbal medicinal plants have been implicated as a remedy for a long time. Among them, herbs and spices are
commonly considered as a safe medicinal drug and have been proved to be effective against certain illness.\textsuperscript{21} Spices herbs are one of the most greatly used antimicrobial substances in foods, not only imparts the flavor and pungent stimuli but also produce antimicrobial property. Nutmeg seed (\textit{Myristica fragrans}) is one of spices herb and it is known potentially to be used as a natural anti-microbial substances in oral care products.\textsuperscript{22} Nutmeg seeds ethanolic extract exhibited antimicrobial activity against enterohemorrhagic \textit{Escherichia coli}, which was found to be highly sensitive to β-pinene.\textsuperscript{23} Therefore, this study was purposed to investigate the antibacterial spectrum of the nutmeg seed ethanolic extract against the staphyloccocal strain i.e \textit{S. aureus} and \textit{S. epidermidis}.

**MATERIALS AND METHODS**

**Plant Materials**

The herb used in the study was nutmeg seeds, obtained from the Spice and Medicinal Plant Research Institute, Bogor, West Java, Indonesia. The plant was identified and determined in Herbarium Bogoriense Botanical Field Bogor Biology-LIPI Research Center.

**Bacterial Strains**

The tested strains of staphylococcus were \textit{S. aureus} and \textit{S. epidermidis}, obtained from the microbiology laboratory of Faculty of Pharmacy, Padjadjaran University, Indonesia.

**Chemical Materials**

The chemicals used were 70\% ethanol, neomycin sulfate (PT. Kimia Farma), normal saline solution, Dragendorf reagents, Mayer reagent, Lieberman - Burchard reagent, vanillin (Merck), sulfuric acid solution (Merck), barium chloride solution (Merck), n-butanol, technical toluene (Brataco), ferric chloride reagent (Merck), and distilled water. The Mueller Hinton Agar (MHA-Oxoid) and Mueller Hinton Broth (MHB-Oxoid) were used as the bacterial growth media.

**Extraction**

Nutmeg seeds were washed using clean water and dried. The dried seeds were powdered and weighed. Then they put into the macerator and soaked with 70\% ethanol. The maceration was done in the room temperature with solvent replacement every 24 h for 3 d. The macerates were collected every 24 h and evaporated until the extract achieved a constant weight.
Phytochemical Screening
The phytochemical contents of nutmeg seed extracts were employed to detect the presence of flavonoids, quinones, steroid/triterpenoids, tannins, alkaloids, saponins and polyphenols using a standard method.[24]

Inoculum Preparation
Bacterial colonies from the slant agar were suspended in 0.95% sterile normal saline. The absorbance of bacterial suspension was measured, then adjusted to get the equal turbidity as the 0.5 McFarland standard.

Antibacterial Activity
The antibacterial activity of nutmeg seed extracts was conducted using an agar diffusion method. A 20 μL of Staphylococcus suspension was poured into the sterile petri dish and suspended in a 20 ml of agar medium (40-45 °C), then it was homogenized and allowed to solidify. The medium then perforated and the extract in different concentration (20, 40, 60, and 80% w/v) were put for 50 μL into the hole. The tests medium was incubated at 37 °C for 18-24 h. The inhibitory diameters zones were measured using calipers.[25]

MIC Determination
The MIC value of the nutmeg seeds ethanolic extract was determined by making serially twofold dilution of the extract in sterile broth medium. The MIC value was defined as the lowest concentration of extract which inhibited the staphylococcal growth. First, the nutmeeg seeds extracts were dissolved in DMSO, then diluted in the MHB medium to achieve the concentrations as follows: 0.3125, 0.625, 1.25, 2.5, 5.0 and 10% w/v. A volume of 10 μl staphylococcal suspensions was inoculated into the tested tubes containing the extract and the broth medium, then they were incubated at 37°C for 18-24 h. The tube with the lowest concentration of the nutmeg seeds extract concentration and did not show the turbidity at that lowest concentration, defined as the MIC value of the extract. As the confirmation, the MIC tubes were dropped and spread on the surface of MHA medium, then incubated at 37°C for 18-24 h. The presence of colonies in the surface of the agar medium was observed.[26]

Comparison Analysis of Antibacterial Activity
The antibacterial activity of the nutmeg seed extracts compared to neomycin sulfate using the agar diffusion method. Both materials were challenged in the same plate. The neomycin sulfate concentration was designed as follows: 20; 40 and 60 μg/ml. Meanwhile for the
extract, 200.000, 400.000 and 600.000 µg/ml. Then the medium was prepared by mixing a 45 µL of bacterial suspension with 45 mL MHA, then the medium was allowed to solidify. The medium was then perforated to make 6 holes in each plate to be filled with a 50 µL of testing concentration and incubated at 37 °C for 18-24 h. The inhibitory diameters were observed, measured and compared. Then the data were plotted to obtain the curve inhibitory against log concentration.\cite{27}

RESULTS AND DISCUSSION

Extraction Result
From 400 g of the dried nutmeg seeds, the obtained extract was 41.277g. Thus the extract rendemen was 10.319%.

Phytochemical Analysis Result
From the phytochemical analysis result, it was found that the nutmeg seeds extracts contained many active antibacterial compounds such as: quinone, tanin, flavonoids, and steroids/triterpenoids. The combination of these compounds may contribute to the medicinal effects of the plant extract.\cite{28}

Antibacterial Activity Result
The antibacterial activity result of the nutmeg seeds extracts against the staphylococcus strains indicated that the extracts were active against both \textit{S. aureus} and \textit{S. epidermidis} with higher activity was against \textit{S. aureus} than to \textit{S. epidermidis}. The compared inhibitory diameters were presented in table 1.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Concentration (%w/v)</th>
<th>Inhibitory Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{S. aureus}</td>
<td>20</td>
<td>12.95±0.0002</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>13.20±0.0001</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>13.70±0.0000</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>14.60±0.0004</td>
</tr>
<tr>
<td>\textit{S. epidermidis}</td>
<td>20</td>
<td>12.20±0.0000</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>12.70±0.0001</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>13.00±0.0001</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>14.20±0.0001</td>
</tr>
</tbody>
</table>

The presence of active compounds in the nutmeg seeds extract was responsible for its antibacterial activity. The tannins are reported to possess antimicrobial activities, and play an
important role in wound healing.\cite{28-30} All the individual active compounds of anthraquinones, tannins, flavonoid, steroids and triterpenes showed antibacterial activity and their synergic effect was also reported.\cite{31}

**MIC of The Extract**

The MIC values of the extract were ranging at $0.156 < x \leq 3.125\%w/v$ for *S. aureus* and $(0.3125 < x \leq 0.625\%w/v)$ for *S. epidermidis*. It was found that MIC values of the extract were lower against *S. aureus* than *S. epidermidis*. These data indicated that the extract was more effective as an antibacterial agent against *S. aureus* infection than *S. epidermidis*.

**Comparative Analysis Result**

Comparative test was conducted to calculate the value of comparative antibacterial activity of nutmeg seeds extract to neomycin sulfate against *S. aureus* and *S. epidermidis*. The inhibitory diameters were displayed in Table 2 and 3. Each of the diameters against different strains was plotted into the equation to obtain equation using linear regression methods. The line equation of neomycin sulfate against *S. aureus* was $y = 2.926x + 8.2169$; as for the extract was $y = 2.958x – 3.8151$. Based on the line equation, the comparative value of the nutmeg seed extracts to neomycin sulfate against *S. aureus* was 1: 11.119. Thus, to produce the same of inhibition zone against *S. aureus*, it took 1 unit of neomycin sulfate, but the extract needed 11.119 units. Meanwhile against *S. epidermidis*, the line equation of neomycin sulfate was $y = 3.149x + 8.0859$; as for the extract was $y = 2.495x – 1.0880$. Based on the line equation, to produce the same diameter inhibition against *S. epidermidis*, it took 1 unit of neomycin sulfate, but the extract needed 15.865 units. Based on the comparative value against *S. aureus* and *S. epidermidis*, it was found that the nutmeg seeds needed more higher unit concentration than that of neomycin sulfate.

**Table 2: Comparison of Inhibition Zone Diameter of Nutmeg Seeds Extracts to Neomycin Sulfate Against S. Aureus.**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Concentration (µg/ml)</th>
<th>Inhibitory Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracts</td>
<td>200,000</td>
<td>11.85±0.0000</td>
</tr>
<tr>
<td></td>
<td>400,000</td>
<td>12.80±0.0004</td>
</tr>
<tr>
<td></td>
<td>600,000</td>
<td>13.25±0.0001</td>
</tr>
<tr>
<td>Neomycin Sulfate</td>
<td>20</td>
<td>12.10±0.0000</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>12.70±0.0002</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>13.55±0.0001</td>
</tr>
</tbody>
</table>

Note: Perforator diameter = 6 mm
Table 3: Comparison of Inhibition Zone Diameter of Nutmeg Seeds Extracts to Neomycin Sulfate Against S. Epidermidis.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Concentration (µg/ml)</th>
<th>Inhibitory Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracts</td>
<td>200.000</td>
<td>12.15±0.0000</td>
</tr>
<tr>
<td></td>
<td>400.000</td>
<td>12.85±0.0002</td>
</tr>
<tr>
<td></td>
<td>600.000</td>
<td>13.35±0.0000</td>
</tr>
<tr>
<td>Neomycin Sulfate</td>
<td>20</td>
<td>12.25±0.0000</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>12.95±0.0002</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>13.80±0.0000</td>
</tr>
</tbody>
</table>

CONCLUSION

It could be concluded that the nutmeg seed extracts can be used as a new strategy to deal with infections caused by strains of staphylococcus, especially *S. aureus* and *S. epidermidis*.

REFERENCES


