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CHALLENGES, STRATEGY AND HEALTH TREATMENT APPROACH TO NUTRITION AND MOLECULAR EPIDEMIOLOGY

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Dr. Sri Wahyuni, S.Pd. M.Kes
STUDY ANTIMICROBIAL ACTIVITY OF ETHANOL EXTRACT CURRY LEAVES (MURRAYA KOENINGII L. SPRENG) AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI

Panal Sitorus¹, Djamidin Manurung², Ernawaty²
¹Departement of Pharmaceutical Biology, Faculty of Pharmacy, Sumatera Utara University
²Polytechnic Health Ministry of Medan
Corresponding Author Email: sitoruspanal@gmail.com

ABSTRACT
Curry (Murraya koenigii L. Spreng) is included in Rutaceae family. Curry leaves are used in herbal medicine and effective antimicrobial agent empirically. The purpose of this study are to extract Murraya koenigii L. Spreng leaves with ethanol, Karacterization, phytochemical screening of simplex and ethanol extract and study its antimicrobial activity of curry leaves extracts, against Staphylococcus aureus and Escherichia coli. Extraction with maceration was carried out by using ethanol 96% at simplex, phytochemical screening and study antibacterial activity curry leaves extracts at Staphylococcus aureus and Escherichia coli conducted as invitro agar diffusion method by using paper disk. The result of simplex and ethanol extract characterization obtained water value 4.63%; 10.50% respectively, water soluble extract value 28.42%; 25.53% respectively, the ethanol soluble extract value 14.83%; 6.21% respectively, the total ash value 8.84%; 4.52% respectively and the acid insoluble ash value 1.13%; 0.85%. The result of phytochemical screening of simplex and extract showed the presence of flavonoids, glycosides, tannins and saponins. The results of study antimicrobial activity of ethanol extract of curry leaves can inhibit the growth of Staphylococcus aureus at concentration of 500 mg/ml, with an effective diameter inhibition zone of 15.03 mm and Escherichia coli at a concentration of 300 mg/ml, with effective diameter inhibition zone of 14.16 mm.

Keywords: Murraya koenigii L. Spreng, Antimicrobial, Staphylococcus aureus, Escherichia coli

Introduction
Plants have an important as medicine. The use of plants as traditional medicine is still used by the people in Indonesia, and traditionally used to treat various types of diseases and effective empirically. The herbs / plants had much success treating some diseases can not be treated with conventional drugs (chemistry). Natural ingredients of traditional medicinal plants Indonesia is still a lot that has not been studied, one of which is a plant greeting Koja/curry (Murraya koenigii L. Spreng). Koja/curry leaves numerous in Aceh province which is called "leaf temurui". Koja/curry leaves used extensively by the community as flavorings cuisine and has a versatile role in traditional medicine. The bark and roots are used to treat venomous animal bites. Leaves to cure dysentery, insect bites, drug injury, diarrhea and vomiting. The leaves and roots are also used traditionally as, anthelmintic, analgesic, curing hemorrhoids, inflammation, itching and can reduce hair loss.

Efficacy of curry leaves in the health field has been widely studied, including anticancer and anti-inflammatory effects, antioxidants antidiabetic and antibacterial. Several researcher had tested the antibacterial essential oil of curry/Koja leaves using the bacterium Escherichia coli, Staphylococcus aureus and Salmonella typhi, the results showed where the essential oil of curry leaves have inhibitory effect on the species of bacterial.
Koja/curry leaves contain secondary metabolites such as tannins, flavonoids and essential oils,\textsuperscript{1,12,23}

Based on the analysis it can be concluded that ethanol extract of curry leaves exhibited the antibacterial activity against \textit{Escherichia coli} and \textit{Staphylococcus aureus}.\textsuperscript{13,20,22,24}

\textbf{Material And Methods}

This study is done by experimental methods. This study involves collecting materials, characterization, phytochemical screening of simplex, extraction and test antimicrobial activity of the extract curry leaves against \textit{Staphylococcus aureus} and \textit{Escherichia coli}. Determination of the antimicrobial activity of extracts of curry leaves performed with agar diffusion method. The principle of this study is to use a solid medium and then the paper disc diameter inhibitory (clear zone) bacteria were measured with calipers,\textsuperscript{9,14}

\textbf{Material}

The materials used in this study were curry leaves (\textit{Murraya koenigii} L. Spreng). distilled water, Nutrient Agar (Difco). The chemical used unless otherwise stated are pro-analysis grade, i.e.: ethanol, n-hexane, mercury (II) chloride, iodine sodium hydroxide, bismuth(III) nitrate, iron (III) chloride, potassium iodide, benzene, a-naphthol, concentrated nitric acid, concentrated hydrochloric acid, sulfuric acid concentrated, lead (II) acetate, acetic acid anhydride, isopropanol, Sodium chloride, chloroform, methanol, Aril alcohol magnesium powders and the microbials were used bacteria \textit{Staphylococcus aureus} ATCC 25923 and \textit{Escherichia coli} ATCC 25 922.

\textbf{Sampling}

The collection of materials was done purposively without comparing with the same plants from the other regions. The samples used are curry leaves (\textit{Murraya koenigii} L. Spreng), taken from Jl. Sempurna kec. Medan Baru, North Sumatera.

\textbf{Identification of Samples}

Identification of Curry/Koja (\textit{Murraya koenigii} L. Spreng) leaves was performed in Bogoriensce Herbarium, LIPI, Jakarta, Indonesia.

\textbf{Extraction}

Extraction was conducted with ethanol by using maceration method. 300 g simplex powder was macerated with 2250 ml of ethanol in a container and let stand at room temperature for 5 days protected from light. Masera was separated with residues. Residues washed with 750 ml of ethanol. All off masera combined and evaporated by rotary evaporator at 40 °C to obtain the extract ethanol, then dried with freeze dryer at -40°C for ± 24 hours and obtain ethanol extract,\textsuperscript{7,8,11}

\textbf{Characterization of Simplex}

Characterization of simplex are to inspection simplex characteristics and study of groups of chemical compounds of ethanol extract curry leaves.\textsuperscript{4,3}

\textbf{Test Antimicrobial Activity of Extract}

The test solution of ethanol extract of curry leaves with various concentrations and test the antimicrobial activity of the extract against \textit{Staphylococcus aureus} and \textit{Escherichia coli}. Determination of the antimicrobial activity of extracts of leaves curry performed with agar diffusion method. The principle of this study is to use a solid medium and then the paper disc diameter inhibitory (clear zone) bacteria were measured with calipers,\textsuperscript{9,10,13,17,20,21}

\textbf{Test Solution Extrac Curry leaves With Various Concentrations}

The ethanol extract weighed 5 g dissolved in dimethyl sulfoxide (DMSO) to 10 ml of the extract concentration is 500 mg / ml, then made further dilution to obtain the extract with a concentration of 400 mg / ml; 300 mg / ml; 200 mg / ml; 100 mg / ml; 75 mg / ml and 50 mg / ml. Then put the paper into the extract discs with various concentrations, let for 30 minutes.\textsuperscript{1,2,9,10}

\textbf{1. Bakteri Staphylococcus aureus}

Inoculum (0.1 ml) put into a sterile petridish, then pour the media Nutrient order of 20 ml with a temperature of (45-50) °C.

Further cup rocked on the surface of the table, so that the media and the bacterial suspension well blended.

After that, implanted paper disk, soaked ethanol extract of curry leaves with various concentrations. Then incubated at 25°C for 48 hours. Furthermore, the diameter of inhibition
zone surrounding paper disk was measured with calipers.

2. *Bakteri Escherichia coli*
Inoculums(0.1 ml) put into a sterile petridish, then pour the media Nutrient order of 20 ml with a temperature of (45-50)°C. Further cup rocked on the surface of the table, so that the media and the bacterial suspension well blended. After that, implanted paper disk soaked ethanol extract of curry leaves with various concentrations. Then incubated at 25°C for 48 hours. Furthermore, the diameter of inhibition zone surrounding paper disk was measured with calipers.

Results and Discussion

Characterization Simplex
The result of simplex and extract characterization shown in Table 1. Below.

Table 1. Characterization of Simplex and Extract

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Result</th>
<th>Extrac</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simplex (%)</td>
<td>Extrac</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>water value</td>
<td>4.63</td>
<td>10.50</td>
</tr>
<tr>
<td>2</td>
<td>water soluble</td>
<td>28.42</td>
<td>55.53</td>
</tr>
<tr>
<td>3</td>
<td>ethanol extract</td>
<td>14.83</td>
<td>65.21</td>
</tr>
<tr>
<td>4</td>
<td>total ash value</td>
<td>8.84</td>
<td>4.52</td>
</tr>
<tr>
<td>5</td>
<td>acid insoluble</td>
<td>1.13</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Phytochemical screening
The result of phytochemical screening of simplex and extract ethanol curry leaves showed the presence of flavonoids, glycosides, tannins and saponins was shown in Table 2. Below.

Table 2. Chemical compounds of curry leaves

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simplex</td>
<td>Extract</td>
</tr>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Glikosids</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroid/TR</td>
<td>+</td>
</tr>
</tbody>
</table>

Test Antimicrobial Activity

The results of test antibacterial activity of ethanol extract of curry leaves against *Staphylococcus aureus* and *Escherichia coli* was shown in Table 3. Below.

Table 3. Antimicrobial Activity of Ethanol Extract

<table>
<thead>
<tr>
<th>NO</th>
<th>Concentration</th>
<th>Regional diameter</th>
<th>Bacterial Growth</th>
<th>extract</th>
<th>Inhibition (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Etanol mg/ml</td>
<td>S.a</td>
<td>E.a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>15,03</td>
<td>17,66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>13,53</td>
<td>15,90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>12,26</td>
<td>14,16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>11,10</td>
<td>13,16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>10,40</td>
<td>11,73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>75</td>
<td>9,40</td>
<td>11,03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>8,26</td>
<td>9,50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Blank</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Specification:
( ) = Average measurement 3x,
( - ) = No results

The test results showed that the antimicrobial activity of the ethanol extract of curry leaves can inhibit the growth of bacteria *Staphylococcus aureus* at concentration of 500 mg / ml with an effective diameter of inhibition area of 15.03 mm and the bacterium *Escherichia coli* at a concentration of 300 mg / ml with a diameter of inhibition area of 14, 16 mm. The minimum inhibitory concentration of ethanol extract can inhibit the growth of *Staphylococcus aureus* at concentrations of 50 mg / ml with inhibition area diameter of 8.26 mm and *Escherichia coli* at a concentration of 50 mg / ml with the diameter of Inhibition zone of 9.50 mm. The antimicrobial activity of a substance to inhibit the growth of microorganisms depend on the extract concentration.

Conclusions and suggestions

Conclusion
From the analysis of the data above it is concluded that:
1. Characteristics powder simplex and ethanol extract curry leaves respectively obtained water value 4.63%; 10.50%, water soluble extract value 28.42%; 55.53%, the ethanol soluble extract value 14.83; 6.21%, the total ash value 8.84%; 4.52% and the acid insoluble ash value 1.13%; 0.85%.
2. Phytochemical screening of simplex powder and ethanol extract of curry leaves showed that the chemical compounds of flavonoids, steroid /triterpenoids, tannins, saponins, and glycosides.

3. The antimicrobial activity of ethanol extract of curry leaves against Staphylococcus aureus and Escherichia coli effectively showed that the antimicrobial activity can inhibit the growth of Staphylococcus aureus at a concentration of 500 mg / ml with an effective diameter of inhibition zone of 15.03 mm and Escherichia coli at a concentration of 300 mg / ml with an effective diameter of the inhibition zone of 14.16 mm.

Suggestions
It is suggested to further research to study ethanol extract of curry leaves against molds.

References


