Isolation and identification of antibacterial compound from diethyl ether extract of *Plantago major* L

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ABSTRACT

*Plantago major* L. is a plant of the Plantaginaceae family which has been investigated on diethyl ether extract generate active spot as an antibacterial. The aim of the investigation was to identify the antibacterial compound from diethyl ether extract of daun sendok. as much 500 gram material was extracted maceration method was obtained 50 gram methanol extract, continued by partition with diethyl ether obtained 19.5 gram dried extract, the extract diethyl ether which isolated by suction column chromatography obtained 3 fractions (F1, F2 and F3). The third fraction (F3) was isolated by column chromatography obtained 2 fractions (3-1 and 3-2). The 3-1 active fraction (inhibit the *Escherichia coli*) isolated by preparative-TLC obtained 2 compounds. Compound 1 was active, single spot and visualization with Lieberman-Buchard and vanillin-sulfate acid. It have a steroid compound.

Keywords: *Plantago major* L., antibacterial, diethyl ether extract.

INTRODUCTION

*Plantago major* L. is widely spread in Asia and the Mediterranean countries; the plant is cultivated extensively in India and Pakistan and adapts to western Europe and subtropical regions.[1,2] Usually wrinkled and contracted leaf and spike, grayish green to dark yellowgreen in colour; when soaked in water and smoothed out, the lamina is ovate or orbicular-ovate, 4–15 cm in length, 3–8 cm in width; apex acute, and base sharply narrowed; margin slightly wavy, with distinct parallel veins; glabrous or nearly glabrous; petiole is rather longer than the lamina, and its base is slightly expanded with thin-walled leaf-sheath; scape is 10–50 cm in length, one-third to one-half of the upper part forming the spike, with dense florets; the lower part of inflorescence often shows pyxidia; roots usually removed, but, if any, fine roots are closely packed.[3] The antidiarrhoeal effects of Semen Plantaginis have been extensively investigated in patients with acute and chronic diarrhoea. An increase in the viscosity of the intestinal contents due to the binding of fluid and an increased colonic transit time (decreased frequency of defecation) were observed in patients treated with the drug.[3,4] The aim of the investigation was identify antibacterial compound from diethyl ether extract of *Plantago major* L.

MATERIALS AND METHOD

Collection of plant material

Fresh plant parts collected from Toli-Toli regency, South East Celebes, the taxonomic identities are preserved at Pharmacognosy Laboratory, Faculty of Pharmacy, Moslem University of Indonesia.

Extraction and isolation of plant materials

Dried leaf (0.5 kg) was macerated with 0.5 liter methanol in five days (this process was done six times), then evaporated which gave 30 gm gummy extract. The gummy extract was dissolved in 80 ml diethyl ether, the diethyl ether fraction was isolated by suction column. The active fraction was continued for isolation using column chromatography method, the column was packed with silica gel G. 60 (Merck) and 0.6 g of the extract was placed on top of the silica and eluted with chloroform:methanol acetate (1:1), (1:50), (1:10) elution in each eluent end. If the solution of fraction
was clear then it was replaced with the other eluent until the last variation. Each fraction from variation of eluent was collected and evaporated at the room temperature.

**MICROORGANISMS**

The following test organisms were used: *Pseudomonas aureginosa, Staphylococcus epidermidis, Vibrio sp.* and *Escherichia coli*.

**Screening for antibacterial activities**

The 10 mg of extracts were dissolved in 0.2 ml dimethyl-sulfoxide (DMSO) and added with 9.8 of GNA medium to a final concentration of 1 mg/mL, bacteria were cultured at 30º C for 24 h.

Antibacterial activity was determined by TLC bioautography method.

**TLC bioautography**

The methanol extract was used for TLC bioautography. Plates were developed with chloroform:methyl acetate (10:1), which separated components into wide range of Rf values. The components were visualized under visible light. Developed TLC plates were carefully dried for complete removal of the solvents and overlaid by nutrient agar seeded with an overnight culture of the bacteria test. The plate was incubated for 24 h at 37º C and then sprayed with Dragendorf and Liebermann-Burchard reagent. Inhibition zones were observed as clear spots against purple background and their Rf values were compared with the reference plate.[5]

**RESULTS AND DISCUSSION**

Result of extraction is shown in Table 1.

The *in vitro* antibacterial activity of diethyl ether extracts and isolate of dried *Plantago major* L. shows that it has activity to inhibit the *Escherichia coli*, as shown in Table 2.

The diethyl ether extract fractionated by suction column give three fractions. The active fraction continued for isolation using column chromatography method is shown in Table 3.

This research used suction column for fractionating the extracts to decrease time for isolation and trough microbial assay to know where the active compound is present and possible to be continued in to the next isolation with column chromatography method.[6]

Results of the antibacterial assay showed that the extract and isolate are only active on the Gram-positive bacteria (*E. coli* and *Vibrio sp.*). The reason for the different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms.

<table>
<thead>
<tr>
<th>Spot</th>
<th>Rf</th>
<th>Color</th>
<th>Rf</th>
<th>Color</th>
<th>Rf</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV 254</td>
<td>UV 366</td>
<td>H2SO4</td>
<td>UV 254</td>
<td>UV 366</td>
<td>H2SO4</td>
</tr>
<tr>
<td>1</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
<td>Green</td>
<td>Blue</td>
<td>Brown</td>
</tr>
<tr>
<td>2</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>Orange</td>
<td>Violet</td>
<td>Green</td>
</tr>
<tr>
<td>3</td>
<td>0.14</td>
<td>0.14</td>
<td>0.14</td>
<td>Green</td>
<td>Yellow</td>
<td></td>
</tr>
</tbody>
</table>

Note: TLC Plate = Silica gel G. 60 F254
Eluent = n-hexane : ethyl acetate (3:1)
Active spot = 2

<p>| Table 1. Result of Isolation 0.5 Kg <em>Plantago major</em> L. using masceration method. |
|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>No</th>
<th>Sample</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Fresh sample</td>
<td>4.905</td>
</tr>
<tr>
<td>2</td>
<td>Dried sample</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Methanol extract</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Diethyl ether extract</td>
<td>19.7</td>
</tr>
</tbody>
</table>

| Table 2. Inhibitory Activity of diethyl ether extracts and isolate of *Plantago major* L. |
|---------------------------------|---------------------------------|
| Bacteria                        | Activity                      |
|                                | Extract | Isolate |
| *Pseudomonas aureginosa*        | –       | –       |
| *Staphylococcus epidermidis*    | –       | –       |
| *Vibrio sp.*                    | +       | –       |
| *Escherichia coli*              | +       | +       |

Note: (-) = No Inhibition; (+) = Inhibition
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**Figure 1.** Result of antimicrobial screening from diethyl-ether extract of *Plantago major* L leaf.

Note: Vsp = *Vibrio* sp, ST = *Salmonella thyphosa*
SE = *Staphylococcus epidermidis*, PA = *Pseudomonas aeruginosa*
EC = *Escherichia coli*, SM = *Streptococcus mutans*
SA = *Staphylococcus aureus*, CA = *Candida albicans*
BS = *Bacillus subtilis*

**Figure 2.** Result of TLC-Bioautography from diethyl ether extract of *Plantago major* L.

**Figure 3.** Result of TLC-Bioautography from third fraction of *Plantago major* L.

**Figure 4.** Result of TLC-Bioautography from Isolate of *Plantago major* L.

Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da.[9]

The Rf values and colour band of the third fraction have been summarized in Table 3. TLC bioautographic assay allowed outlining the chemical profile of the third fraction thus detecting the active substances that presented antimicrobial activity. The results of this assay revealed that compounds eluted at Rf 0.19, exhibited strong antibacterial activity against *E. coli* therefore, suggesting the presence of antimicrobial active substances. The phytochemistry assay on the active spot with Lieberman-Buchard reagent and vanillin-sulfate acid showed that the active compound is steroid.

**CONCLUSION**

Based on these results, it is possible to conclude that *Plantago major* L has a strong antibacterial activity against bacteria and the extract can be used as an antibacterial agent for diarrheal effects of *E. coli*. 
REFERENCES