INTRODUCTION

Zingiberaceae is one of the common plants in Indonesia which used as traditional medicine, whereas subfamily Zingiberidae distributed in Indo-Malaysia [1]. The essential oil of Zingiberaceae have been studied as an antimicrobial, larvicide, and repellant [2, 3]. In addition, previous studies have been conducted, and reported that sesquiterpenoid diarylheptanoid were compounds commonly found in Zingiberaceae plants [4, 5, 6]. The Zingiberaceae known as a source of flavonoids and often contain tannins. Matsuda et al (2002) isolated the flavonoid compound from Hedychium coronarium [7] and the rhizome of H. spicatum [8]. Previous research assumed that there was correlation between antimicrobial activity and flavonoid content. The antimicrobial activity of the alcoholic and aqueous extract of ginger (Zingiberaceae) with the presence of (6)-gingerol that argue as active composition, were showed optimal activity against Escherichia coli, Pseudomonas aeruginosa and Candida albicans [9]. The objective of this research were to study antimicrobial properties and flavonoid content of four Zingiberaceae plants which obtained randomly at some place on West Java – Indonesia. The species were Nicolaia hemisphaerica (Blume) Horan, Horstedtia pininga (Blume) Valet, Amomum hochreutineri Val. and Hedychium roxburghii Blume.

MATERIALS AND METHODS

Chemicals

Muller Hinton Broth (MHB) (Flucda ®), Muler Hinton Agar (MHA) (Sigma-Aldrich®), quercetin (Sigma-Aldrich®), tetracycline, nystatine, methanol, n-hexane, ethyl acetate, ethanol 95%, chloroform, aquadest, toluene, hydrochloride acid, sulfuric acid, magnesium powder, amyl alcohol, Fe(III)chloride, sodium sulfate anhydride, sodium acetate, gelatine, sodium hydroxide, kalium iodide, aluminium chloride. All other reagents were analytical grade.

Rhizome samples

The rhizomes of Nicolaia hemisphaerica (Blume) Horan, Horstedtia pininga (Blume) Valet, Amomum hochreutineri Val. and Hedychium roxburghii Blume were collected from some area in West Java – Indonesia. The rhizomes were thoroughly washed with water, wet sortation, sliced, and grinded into powder.

Extract preparation

Extraction was conducted by reflux method using ethanol as a solvent then evaporated with rotavapor (Buchi ®).

Determination of total flavonoid content

Total flavonoid content was measured using modified method adapted from Chang [10]. Preparation of standard solution: 1000 µg/mL of quercetin (in methanol) diluted to 20, 40, 60, 80, 100, 120, and 140 µg/mL. Crude drugs (0.8 g) were extracted by maceration method with 10 mL ethanol 24 hours. Extract was evaporated then diluted volumetric with 5 mL of methanol. Extract or standard solution (0.5 mL), 1.5 mL of methanol, 0.1 mL of AC13% (in aquadest), 0.1 mL of sodium acetate 1 M and 2.8 mL aquadest were incubated at room temperature in 30 minutes. The absorbance was fixed at λ 415 nm. The total flavonoid content was measured as percentage of total g quercetin equivalents per 100 g extract (g QE/100 g).

Microorganism

The microorganism were Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6633, Pseudomonas aeruginosa ATCC 9027, Escherichia coli ATCC 8939, Candida albicans ATCC 10231 were used for determined antimicrobial activity. Each microorganism stock cultures were incubated overnight at 37°C on Nutrient Agar for bacteria and 250°C on Sabouraud Dextrose Agar for fungal.

Preparation of inoculum

A loopful of inoculum inoculated into 10 mL Muller Hinton Broth. The broth suspension was incubated overnight at 37°C for bacteria and 250°C for fungal.

Antimicrobial activity test

Antimicrobial activity was measured using microbroth dilution method adapted from CLSI [11]. The suspension for inoculation was...
prepared by diluted the broth culture with medium solution to obtained 0.5 McFarland (5 x 105 CFU/mL) suspension then diluted 1:20 with medium. When 0.01 mL of this suspension was inoculated into 96 well microtiter plate. The final concentration of extracts were 1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.25 μg/mL, 15.6 μg/mL, 7.8 μg/mL. Tetracycline, ciprofloxacin, nystatine and ketoconazole were used as positive controls.

RESULTS
The total flavonoid content of four Zingiberaceae plants were measured by extrapolation to the standard curve calibration of quercetin. The equation of standard curve was y = 0.007x + 0.021; R²= 0.959. The results showed different flavonoid content of each species in range of 0.020-0.162% (w/w extract). The highest yield was shown by Hedychium roxburghii rhizome extract (0.162%), followed by Anomum hochreutineri (0.076%) of Nicolaia hispensis (0.071%) and Hornstedtia pininga (0.04%).

Table 1: Total flavonoid content of four Zingiberaceae plants

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Flavonoid (%)</th>
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<tbody>
<tr>
<td>N. hispensis</td>
<td>0.071 ± 0.006</td>
</tr>
<tr>
<td>H. pininga</td>
<td>0.040 ± 0.003</td>
</tr>
<tr>
<td>A. hochreutineri</td>
<td>0.076 ± 0.007</td>
</tr>
<tr>
<td>H. roxburghii</td>
<td>0.162 ± 0.004</td>
</tr>
</tbody>
</table>

The antimicrobial activity of four Zingiberaceae plants showed various minimum inhibitory concentration and broad activity against test-microorganism. The results shown on the Table 2.

Table 2: Minimum Inhibitory Concentration of Four Zingiberaceae plants

<table>
<thead>
<tr>
<th>Species</th>
<th>Minimum Inhibitory Concentration [MIC] (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>N. hispensis</td>
<td>1000</td>
</tr>
<tr>
<td>H. pininga</td>
<td>500</td>
</tr>
<tr>
<td>A. hochreutineri</td>
<td>500</td>
</tr>
<tr>
<td>H. roxburghii</td>
<td>500</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.2</td>
</tr>
<tr>
<td>Nystatine</td>
<td>-</td>
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</tbody>
</table>

DISCUSSION

Flavonoids are the secondary metabolite which is increasingly becoming the subject of medical research. They have been shown to have many pharmacological properties, including antioxidant activity, antiallergic activity [12], anti-inflammatory activity, oestrogenic activity, antimicrobial activity [13],[14], vascular activity and cytotoxic activity [15]. Many folk medicine was reported containing flavonoids as the principal physiologically active constituent. For example, the plant Tagetes minuta was used traditionally to treat infectious diseases in Argentina, it was known contain quercetagenin-7-arabinoisyl-galactose [16].

Actually, when reports of the antibacterial activity of flavonoids were compared, the results appeared widely conflicting. Recently, a set of guidelines was published for standard broth microdilution methods [16]. Among all the tested rhizome of four Zingiberaceae, H. roxburghii showed the highest content of total flavonoid (0.162 ± 0.004%) followed by A. hochreutineri (0.076%). N. hispensis (0.071%) and H. pininga (0.04%). The flavonoid compound of those plants have not identified yet, but other plants with the same genera (0.04%) followed by N. hemisphaerica (0.071%) and H. pininga (0.04%).

In this study, antimicrobial test results of four Zingiberaceae by broth microdilution method showed in line correlation with total flavonoid content whereas H. roxburghii Bl. showed a broad antibacterial spectrum and the strongest activity especially against P. aeruginosa (MIC 62.5 μg/mL).

CONCLUSION
In the present study, ethanolic extract of four Zingiberaceae showed an antimicrobial activity with various potency. Hornstedtia pininga, Anomum hochreutineri and Hedychium roxburghii showed strong activity against Bacillus subtilis (MIC at 50 μg/mL). The strongest activity against E. coli were showed by H. pininga and H. roxburghii at MIC 31.25 μg/mL. Whereas, the strongest antimicrobial activity against P. aeruginosa was H. roxburghii (MIC 62.5 μg/mL). In line with total flavonoid content, the highest yield was obtained by H. roxburghii rhizome extract (0.162%), followed by A. hochreutineri (0.076%), Nicolaia hispensis (0.071%) and H. pininga (0.04%).

CONFLICT OF INTERESTS
Declared None

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REFERENCES


