Antibacterial and Phytochemical Analysis of Two Plants Menispermaceous Family

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ABSTRACT
The failure of antibiotic treatment due to resistant bacteria made emerging cases in this century. This situation needs new antimicrobial from nature to cure it. Coscinium fenestratum (CF) and Fibraurea tinctoria (FT), both plants from Borneo usually were used by local ethnic to treat various diseases caused bacteria. Hence, our study to determine antibacterial activity of CF and FT stem and finding secondary metabolite which could inhibit or kill bacteria. This study used extract ethanol of the stem plants, MDA (ATCC 43300), MRSA (ATCC 25923), and local isolates of MDR E. coli. Antibacterial activity was measured by Kirby-Bauer Method, MICs and MBCs, while TLC for secondary metabolites Value of inhibition zone for extract of CF based on concentration was MSSA (8.1±0.0), MRSA (8.1±0.0), MDR E. coli (8.1±0.0) to 1mg/ml; MSSA (8.1±0.0), MRSA (10.0±0.1), MDR E. coli (8.1±0.0) to 2 mg/ml; MSSA (12.1±0.0), MRSA (12.1±0.0), MDR E. coli (12.0±0.0) to 8 mg/ml. While for FT was MSSA (8.1±0.0), MRSA (8.0±0.0), MDR E. coli (8.0±0.0) to 1 mg/ml; MSSA (8.0±0.0), MRSA (10.0±0.1), MDR E. coli (8.0±0.0) to 2 mg/ml; MSSA, MRSA, MDR, E. coli to 4 mg/ml; MSSA, MRSA, MDR E. coli to 8 mg/ml. Meanwhile, MIC and MBC results for extract of CF was MIC (25 mg/ml), MBC (50 mg/ml) to MSSA; MIC (62.5 mg/ml), MBC (12.5 mg/ml) to MRSA, MIC (25 mg/ml), MBC (25 mg/ml) to MDR E. coli; while for FT was MIC (25 mg/ml), MBC (25 mg/ml) to MSSA; MIC (3.13 mg/ml), MBC (6.25 mg/ml) to MRSA; MIC (25 mg/ml), MBC (25 mg/ml) to MDR E. coli and then both extracts had retention factor 0.32 and 0.42 for secondary metabolites. Both plants had antibacterial effect but FT had stronger effect than another, therefore, it could be candidate for new antimicrobial.
Keywords: Coscinium fenestratum, Fibraurea tinctoria, antibacterial, secondary metabolite.

INTRODUCTION
Multidrug resistant to various infection agent has emerged cases this decade. The failure of antimicrobial treatment due to new super bugs bacteria, such as Methicillin Resistant Staphylococcus aureus and Multiple Drug Resistant Escherichia coli. The condition leads new treatment of antimicrobial from nature because use plant as resources against bacteria increasing the number of livestock free from chemical antibacterial residual.1,2
In Indonesia, a traditional herb has been known as Jamu and many plants from its forests have been used for it treating various infectious diseases.3 Kalimantan, one of islands in Indonesia have traditional herbs which are used by Dayak ethnicities treating diseases, for instance Coscinium fenestratum (Goeth.) Celebr Fibraurea tinctoria Lour. These plants are family of Menispermaceous and have been known as yellow-wood-climber (akar kuning).2 The Dayak ethnic in Kalimantan could not the difference between those plants. This community only know that stem of these plants has yellow color and using as medicine to cure diseases, such as diarrhea, dysentery, malaria, skin disease, diabetes and jaundice.2,4,5 Thus, both of them have ability to cure various diseases making scientists interested to explore these plants. The scientists suggest these plants can act as antibacterial to inhibit or kill germs. One of scientists was interested to further study was Andreas et al (2018) and they found, the leaves and roots of F. tinctoria (FT) had ability as antimicrobial toward Enterococcus faecalis but the extract of ethanol this plant could not inhibit Staphylococcus aureus and Pseudomonas aeruginosa. Andreas et al (2018) study was dissimilar with Zalizar, et al (2019). They found the combination stem and root of FT had ability to inhibit S. aureus and E. coli growth and they found not only FT but C. fetenum (CF) can act like antibacterial too. The stem of CF extract can inhibit S. aureus and Streptococcus pyogenes.4 The scientist assumed that the ability of those tribe plants was due both containing chemical compounds were called as secondary metabolite. Secondary metabolite is the chemical compounds formed during normal metabolic process. There are several classes including alkaloids, tannins, flavonoids, coumarins, glycosides, phenols, terpenoids and terpenes which can act as agents to inhibit or kill germs; therefore, it is important knowledge to know these secondary metabolites can be used for therapeutic purposes because phytochemical present are desirable for the discovery a new drug toward multidrug resistant antibacterial cases.7 Hence, our study to determine antibacterial activity of stem of CF and FT and secondary metabolites from both extracts which could inhibit or kill MSSA, MRSA and MDR E.coli bacteria.

MATERIAL AND METHODS
Collection and Identification of Plant Materials
The stem of the plants were collected from Samboja Kutai Kartanegara East Borneo. Identification of C. fenestratum (No. 77/UN17.4.308/LL/2016) and F. tinctoria (No. 80/UN
Preparation of Plant Materials and Extracts

Both stems of plants were washed with distilled water, dried at the temperature room, followed by drying in the oven. Making the simplisia using a mechanical grinder and then, carried in cleaned airtight bottles for maceration in 60% ethanol for three days. Every day whipped all bottles with orbital shaker at 2 rpm for 10 minutes and repeated it three times. The extracts filtered with micro fiberglass filter in the vacuum pump, and then continued with freeze-drying process. Plant extracts obtained stored at 4°C.

Bacteria Strains

Bacterial strains used in this study are MSSA (ATCC 43300), MRSA (ATCC 25923), and local isolates of MDR E. coli (Isolate from Abdul Wahab Syahranie Hospital). All these strains cultured in Brain Heart Infusion broth at 37°C for 24 hours. The concentrations all bacteria cultures were measured by spectrophotometry (Becton-Dickinson, USA) until had 0.5 McFarland (1.5 x 10^-8 CFU/ml).

Disc Diffusion Method (Kirby-Bauer Method)

Disc diffusion method was based on method of Ifesan et al. (2010). Ten microliters of the extracts dissolved in ethanol and added to sterile filter paper discs (Oxoid™, UK). The discs dried at 70°C in oven overnight. The plates of Mueller-Hinton agar (Merck, USA) applied with 200 uL culture of bacteria and then, the discs contained extracts seeded on those plates. The Discs of Methicillin 10 µg (Oxoid™, UK) and oxacillin 1µg (Oxoid™, UK) used as positive controls.

The plates were incubated at 37°C for 24 hours. These experiments performed in three times duplicate and the means of the diameters of the inhibition zones were calculated by vernier caliper (Merck, USA).

Evaluation of MICs and MBCs

Evaluation MICs was performed by method of Shaheen et al. (2015). The 96-well microplates incubated at 37°C for 24 hours. MICs were defined as the lowest concentration of highest dilution of plant extracts at which no visible bacterial growth was observed in the microliter wells after 24 hours and still no growth after further 24 hours was regarded as MBCs. These experiments performed in three times duplication.

Bioautography with TLC (thin layer chromatography)

Bioautography with TLC based on method of Suleiman et al. (2010). First, plant extracts loaded on TLC plates in a narrow band and were eluted by methanol. Second, the developed plates dried using laminar flow cabinet overnight and then, the plates mounted on bacteria cultures grown on Mueller-Hinton agar. Finally, colourless bands showed secondary metabolites that inhibited the growth of tested organisms. Visualization is usually carried out by spraying plates with MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) for clearer results and was analysed by Retention factor (RF) measurement. The formula of RF score was distance of sample divided to distance of the solvent.

DATA ANALYSIS

Antibacterial activity was described as the mean ± standard error (Mean±S.E.M.) and statistical analysis was carried out by linear Regression (SPSS Statistics 23) and the significant level was p<0.05.
RESULT

![Fig 1: Inhibition Zones of CF and FT](image)

Note: Inhibition zones are presented as mean of triplicates ± Standard Error. Inhibition Zones include disc diameter (6 mm).

Abbreviations: EtOh (Ethanol), Met (Methicillin), Ox (Oxicillin)

In the figure 1 showed the bacterial activity of extract stems of CF and FT by Kirby - Bauer method against MSSA, MRSA, and MDR E.coli. FT had been greater bacterial activity (15±0.1) against MRSA at 8 mg/ml concentration than CF (12.1±0.1), whatever; antibacterial activity of FT had lower ability against MSSA bacteria (10.1±0.0) than CF (12.1±0.0) at the same concentration as well. According to these data, both plants can be used to develop new antibacterial, due to both had inhibition zones higher at 4 mg/ml and 8 mg/ml concentrations than Methicillin 10 µg/ml and oxacillin 1 µg/ml against MSSA, MRSA, and MDR E. coli.
Figure 2: A. Inhibiting zone (mean ± S.E.M) of CF and FT towards MSSA, MRSA and MDR E. coli
B. Inhibiting zone (mean ± S.E.M) of different concentration of plant extract (CF and FT) towards MSSA, MRSA and MDR.

On the figure 2A showed, FT had inhibited ability stronger than CF towards MRSA, but its ability lower than FT against MSSA. While, no significantly differ inhibiting ability between CF and FT against MDR E. coli.

On the figure 2B showed the graphic between concentration of both plants and inhibition zone of bacteria. Its showed, the graphic of MRSA increase sharply than MDR E. coli. Its line steadily on 1 mg/ml to 2 mg/ml of concentration and then increase slightly. On contrary graphic was made by MSSA. It had been making static line from 1 mg/ml to 2 mg/ml of concentration and increase slightly until 4 mg/ml of concentration and then, the constant line in the last.

Based on the data, the antibacterial effect of MRSA increased by the concentration increasingly. The data similar to linear regression analysis result; wherein, there was significantly difference between inhibition zone of FT and CF against MRSA and MSSA at 8 mg/ml concentration (p< 0.001). Although, it was differing to MDR E. coli due to its significant result at 4 mg/ml concentration with p= 0.12 (p<0.05).

Table 1:

<table>
<thead>
<tr>
<th>Groups</th>
<th>MSSA MIC (mg/ml)</th>
<th>MSSA MBC (mg/ml)</th>
<th>MRSA MIC (mg/ml)</th>
<th>MRSA MBC (mg/ml)</th>
<th>MDR E.coli MIC (mg/ml)</th>
<th>MDR E.coli MBC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract of CF</td>
<td>25</td>
<td>50</td>
<td>6.25</td>
<td>12.5</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Extract of FT</td>
<td>25</td>
<td>25</td>
<td>3.13</td>
<td>6.25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

In the table 1, both bacteria MSSA and MDR E. coli did not growth at 25 mg/ml concentration of CF, likewise FT could inhibit MSSA and MDR E. coli at the same concentration as well. Meanwhile, MRSA bacteria did not growth at 6.25 mg/ml concentration of CF and 3.13 mg/ml concentration of FT. This condition confirmed that 25 mg/ml was minimum inhibitory concentration (MICs) for MSSA and MDR E.coli for both extract and 6.25 mg/ml was minimum inhibitory concentration (MICs) for MRSA bacteria in CF extract and 3.13 mg/ml FT extract.

On contrary with MBCs, bacteria of MDR E.coli had the same concentration between MICs and MBCs on both CF and FT as well as bacteria of MSSA had a same value between MICs and MBCs on FT. Whereas, MSSA bacteria had MBCs value as 0.5 mg/ml on CF MRSA had 1.3 mg/ml on CF and 0.6 mg/ml on FT. Even though CF and FT had susceptibility to inhibit and kill the MRSA bacteria.
bacteria, but FT had the lower concentration could inhibit and kill MRSA bacteria than CF.

Figure 3: Secondary metabolite of CF and FT

According to the figure 3, CF had the similarity of retention factor with FT and its value was 0.32 and 0.42 for both of the plants. Based on Supattra study, the secondary metabolite which having these retention factor values were alkaloid and it has ability as antibacterial.

**DISCUSSION**

As traditional folk medicinal plants, CF and FT are widely used to cure various diseases in Dayak community. The synonym of *C. fenestratum* is Coscinium maingayi Pierre; Coscinium miorepalum Diels; Coscinium walichianum Miers; Coscinium wightianum Miers ex Diels; Menispernum fenestratum Gaertn; and the synonym of *F. tinctoria* is Cocculus fibraurea DC; Fibraurea chloreleucu Miers; Fibraurea manipuresis Brace ex Diels; Fibraurea laxa Miers; Fibraurea fasciculata Miers; Fibraurea irotteri Watt ex Diels; Menispernum tinctorium Spreng. Both extract of these plants had antibacterial properties which might inhibit or kill MSSA, MRSA and MDR E. coli bacteria.

Based on the result, both ethanol extract of plants had antibacterial activity using Kirby Bauer method. These plants had strong antibacterial activity toward MSSA, MRSA and MDR E. coli at 4 mg/ml and 8 mg/ml concentration due to inhibition zone ≥ 10 mm, but only MRSA could be inhibited by these extracts at 2 mg/ml concentration. This condition could be happened because gram-positive have thicker layer than gram-negative, but it does not have lipopolysaccharide (LPS); thus, gram positive is more sensitive than gram-negative bacterial. On contrary result was happened between MSSA and MRSA. The scientists assumed, the different genes evolution was involved between these bacteria and seemed one of the genes which responsibility to resistance was mecA gene. The mecA could be found in MRSA bacterial but couldn’t find in MSSA. Besides mecA gene, there are several genes, such as fem, IIm and sigB are involved and both extracts can be interacted to them and all those genes might be influenced the proliferation and differentiation of MSSA and MRSA cells.

Capability of antimicrobial to inhibit or kill bacterial was depended on MICs and MBCs value. The antibacterial or antimicrobial only had bacteriostatic effect, when it only inhibited bacteria growth. But, when it could kill the bacterial, its act as bactericidal. Its effect could be found when the MBCs value had no greater fourfold than MICs value. In the meanwhile, our study showed, both plants had bactericidal effect against MSSA, MRSA and MDR E. coli and the strongest ability was MRSA. The inhibition zone, MICs and MBCs result were showed these plants had bactericidal effect; However, it is very important to know the chemical compounds containing these plants which could explain inhibiting or killing ability of bacteria. Phytochemical analysis of both stems was found two secondary metabolite which had Retention factor (Rf) 0.32 and 0.42 and those were suggested alkaloid. The alkaloids are containing one more nitrogen atom. They are classified based on their chemical structure or natural origin and some of them have antibacterial activity through inhibiting enzyme, affecting cell division, respiratory inhibition, membrane disruption and affecting virulence genes of bacteria.

Based on chemical structure, alkaloids are divided into protoberberines, quaternary alkaloid, quaternary protoberberine. Berberine, palmatine and jatrorrhizine are protoberberine alkaloids. These alkaloids had moderate, slow and selective ability as antibacterial with a low
toxicity. It used to therapy various diseases due to bacteria and until now, no allergic cases ever reporting. Those are effective inhibiting or killing resistant bacteria through modifying target on surface bacteria, inhibiting enzyme of bacteria, modifying membrane of bacteria, and inhibiting efflux pump on surface bacteria.\textsuperscript{23} Supattra (2001) found retention factor 0.32 and 0.42 were palmatine and jatrorrhizine.

Siwon et al (1980), reported these alkaloids could inhibit the bacteria Staphylococcus aureus, Bacillus subtilis, Penicillium luteum and Candida albicans. Even though they alone were different to every alkaloid; for instance, the bacteriostastic effect of protoberberine alkaloid of CF against S. aureus.\textsuperscript{23,24,25} They found the highest activity was berberine and then was continued by palmatine and jatrorrhizine (berberine > palmatine > jatrorrhizine). Similarly, the Luo et al, 2013 study on MRSA bacteria. They found, berberine showed the highest antibacterial activity and then was continued by optisine, jatrorrhizine, palmatine and the last was epiberberine (berberine > optisine > jatrorrhizine > palmatine > epiberberine).

Hence, further study might be need to explore these plants, especially FT because it had the strongest ability as antibacterial against MRSA and could act bactericidal based on MICs and MBCs result.

**CONCLUSION**

Based on our study, CF and FT had antibacterial effect but FT had stronger effect than another; therefore, it could be candidate for new antimicrobial.

**CONFLICT OF INTEREST**

All authors declared no conflict of interest.

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