Antibacterial Activity of Ethanol Extract of Kemuning (Murraya Paniculata) Against Klebsiella pneumoniae ESBL by In Vitro Test

Illona Okvita Wiyogo¹, Pepy Dwi Endraswari², Yuani Setiawati³

¹Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia
²Department of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia
³Department of Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia

Received: 07th August 2018; Revised: 08th March 2021

ABSTRACT

Klebsiella pneumoniae extended-spectrum β-lactamase (ESBL) was one of the microorganism that cause nosocomial infection which resistant to beta-lactams antibiotics. Orange Jessamine (Murraya paniculata) was traditional medicine which believed has antibacterial components, such as: flavonoids, alkaloids, essential oils, coumarins, terpenoids, tannins, and saponins. In the previous studies, there was antibacterial activity in ethanolic extract of Murraya paniculata againsts E.coli, K.pneumoniae, S.typhi, E.faecalis, P.aeruginosa, S.flexneri, S.aureus, and S.sonnei with concentration 200 mg/mL. There has not experiment about ethanolic extract of Murraya paniculata against Klebsiella pneumoniae ESBL yet. The aim of this study was to find out the in vitro antibacterial activity of ethanol extracts of Murraya Paniculata against Klebsiella pneumoniae ESBL Broth dilution method with concentration 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12,5 mg/mL, 6,25 mg/mL, and 3,125 mg/mL were used for the determination of the Minimal Inhibitory Concentration (MIC). While the Minimal Bacterial Concentration (MBC) was assessed using streaking method in Nutrient Agar Plate. The highest concentration in this study was obtained from 100 g of Murraya paniculata leaves dissolved in 500 mL of 40% ethanol. The study was carried out 4 times replication. At the time of the sterility test extract, germ growth appeared on Nutrient Agar Plate media, so the extract was filtered before being used for research. After incubation at 37 °C for 24 hours, growth of bacterial colonies on all agar plates was observed. The concentration of the ethanol extract of Murraya Paniculata (200 mg/mL) did not inhibit the growth of Klebsiella pneumoniae ESBL. The ethanol extracts of Murraya paniculata in concentration 200 mg/mL had no antibacterial activity against Klebsiella pneumoniae ESBL.

Keywords: Antibacterial, ethanol extracts, Klebsiella pneumoniae, ESBL, Murraya paniculata leaves

ABSTRAK

Klebsiella pneumoniae Extended-spectrum β-lactamase (ESBL) merupakan salah satu mikroorganisme yang menyebabkan infeksi nosokomial yang resisten terhadap antibiotik beta-laktam. Oranye Jessamine (Murraya paniculata) adalah tradisional obat yang dianggap memiliki komponen antibakteri, seperti: flavonoid, alkaloid, minyak esensial, kumarin, terpenoid, tanin, dan saponin. Dalam studi sebelumnya, ada antibakteri aktivitas ekstrak etanol Murraya paniculata melawan E.coli, K.pneumoniae, S.typhi, E.faecalis, P.aeruginosa, S.flexneri, S.aureus, dan S.sonnei dengan konsentrasi 200 mg/mL. Belum ada eksperimen tentang ekstrak etanol Murraya paniculata terhadap Klebsiella pneumoniae ESBL. Tujuan dari penelitian ini adalah untuk mengetahui aktivitas antibakteri in vitro ekstrak etanol Murraya Paniculata terhadap Klebsiella pneumoniae ESBL. Metode pengenceran kuldhu dengan konsentrasi 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12,5 mg/mL, 6,25 mg/mL, 3,125 mg/mL digunakan untuk penentuan Minimum Inhibitory Concentration (MIC), sedangkan Minimal Bacterial Concentration (MBC) diniat menggunakan metode goresan pada Pelat Agar Nutrien. Konsentrasi tertinggi dalam penelitian ini diperoleh dari 100 g daun Murraya paniculata yang larut dalam 500 mL etanol 40%. Penelitian dilakukan 4 kali replikasi, dimana pada saat ekstrak uji sterilitas pertumbuhan kuman muncul pada media agar nutrien agar, sehingga ekstrak disaring sebelum digunakan untuk penelitian. Setelah inkubasi pada 37 °C selama 24 jam, pertumbuhan koloni bakteri pada semua

* Corresponding Author: illonawi@gmail.com
sehingga piring diamati. Konsentrasi ekstrak etanol Murraya Paniculata (200 mg/mL) tidak menghambat pertumbuhan Klebsiella pneumoniae ESBL. Ekstrak etanol Murraya paniculata dalam konsentrasi 200 mg/mL tidak memiliki aktivitas antibakteri terhadap Klebsiella pneumoniae ESBL.

**Kata Kunci:** Anti bakteri, Ekstrak Etanol, Klebsiella pneumoniae, ESBL, Daun Murraya paniculata

**How to cite:** Wiyogo, I., Endraswari, P., & Setiawati, Y. (2021). Antibacterial Activity of Ethanol Extract of Kemuning (Murraya Paniculata) Against Klebsiella pneumoniae ESBL by In Vitro Test. Indonesian Journal of Tropical and Infectious Disease, 9(2) 102-107

**INTRODUCTION**

The percentage of nosocomial infection in Haji Adam Malik Hospital, Medan in 2010 is 6-16% and the mean is 9.8%. The most frequently detected infection is Nosocomial Pneumonia (both ventilator and non-ventilator associated), and followed by urinary tract infection and central venous catheter associated bloodstream infections respectively. Ventilator-associated Pneumonia (VAP) is the most common nosocomial infection among critical patients.

Nosocomial infection is handled differently from the non-nosocomial one since nosocomial infection is generally due to Multidrug-resistant bacteria. In developing countries, antibiotics are often used in irrational dose, hence the increased prevalence of antibiotic-resistant bacteria in hospitals. The prevalence of Imipenem-resistant Acinetobacter, Imipenem-resistant Paeruginosa, and Oxacillin-resistant S.aureus are 67.3%, 27.2% and 82.1% respectively. Several bacteria are categorized as multidrug-resistant. The bacteria’s high level of resistance might limit therapy options.

Bacteria producing extended-spectrum β-lactamase (ESBL) cannot be overcome with penicillin, cephalosporin, and monobactam aztreonam, such as several K. pneumoniae strains. The infection case of K. pneumoniae ESBL in the group of nosocomial infection is 11 times higher than those of the community-acquired infection group.

Indonesia is a tropical country with abundant medicinal plants, one of which is orange jessamine (Murraya paniculata). Medicinal plants generally contain phenolic compounds, i.e. phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, etc. The leaves of orange jessamine (Murraya paniculata) contain bioactive compounds which are secondary metabolites, such as alkaloids, flavonoids, saponins, terpenoids, and tannins. Since 1970, flavonoids and coumarins have been isolated from Murraya paniculata. Evaluations on the synthesis of nitro coumarins with or without the substitution of methyl or methoxy group has shown antibacterial activity against Staphylococcus aureus, Escherichia coli and Candida albicans strains.

Other studies report that Murraya paniculata has many benefits including anti-platelet aggregation, antiamoebic, anti-giardial, insecticide, pain relief, antidiabetic, antioxidant, antifungal, lipoxygenase and respiratory burst inhibitor. This study aims to test antibacterial activity of orange jessamine extract against Multidrug-Resistance (MDR) bacteria, Klebsiella pneumoniae producing ESBL.

**METHODS**

This is a quasi-experimental study with Convenience Post Test Controlled Design, aimed to find out the antibacterial activity of the leaves of orange jessamine against Klebsiella pneumoniae ESBL with dilution method. The orange jessamine (Murraya paniculata) leaf extract were obtained from Materia Medica Laboratory, Batu, while antibacterial activity was tested in Microbiology Laboratory of Faculty of Medicine Universitas Airlangga.

The independent variable is ethanol extract of orange jessamine (Murraya paniculata) leaf extract concentration, while the dependent
variable is inhibition effect of *Klebsiella pneumoniae* ESBL bacteria growth in each tube containing concentration of ethanol extract orange jessamine leaf. The control variable are temperature and incubation time of sensitivity test with dilution method.

The suspension treatment groups of *Klebsiella pneumoniae* ESBL was exposed to ethanol extract orange jessamine leaf. The groups were T1 (100%), T2 (50%), T3 (25%), T4 (12.5%), T5 (6.25%), T6 (3.125%) and T7 (1.5625%). The control groups consisted of K1 (liquid medium and bacteria) and K2 (liquid medium and ethanol extract orange jessamine leaf). Minimum Inhibitory Concentration (MIC) is the lowest concentration that is still able to inhibit bacterial growth. Whereas, Minimum bactericidal concentration (MBC) is the lowest concentration that is able to kill bacteria. A further observation to determine MBC can be conducted upon the obtaining of growth inhibitory effect.

The test for antibacterial activity in ethanol extract orange jessamine leaf against *Klebsiella pneumoniae* ESBL is conducted with dilution method to determine MIC and MBC, which are analyzed descriptively and statistically using Analysis of Variance (Anova).

**RESULT**

This study used orange jessamine leaf extract obtained through maceration method. The highest concentration in this study was obtained from 100 g of *Murraya paniculata* dissolved in 500 mL of 40% ethanol. The compared extract concentrations were 200 mg/mL, 100 mg/mL, 50 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.125 mg/mL. In this study, replications were carried out four times. During extract sterility test, there was bacterial growth in the medium Nutrient Agar Plates. Thus, the extract was filtered prior to the use in this study (Table 1).

The use of dilution method was aimed to assess the MIC. There were seven tubes with different concentration of extract ethanol of *Murraya paniculata* leaves and two tubes as the positive and negative control. The positive control tube contained bacteria and liquid Mueller Hinton Broth (MHB), while the negative one contained extract and liquid MHB. From all the conducted replications, all tubes were unable to identify as the extract color tended to appear dark (Figure 1). Thus, streaking was carried out on Nutrient Agar Plates to directly observe if there was any inhibition in the growth of *Klebsiella pneumoniae* ESBL bacteria by extract ethanol of *Murraya paniculata* leaves. After incubated in the temperature of 37°C for 24 hours, bacterial growth appeared in all Nutrient Agar Plates (Figure 2). The adding of extract up to the highest concentration showed that there was still bacterial growth in Nutrient Agar Plates

<table>
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<tr>
<th>Replication</th>
<th>Extract Concentration (mg/mL)</th>
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<tr>
<td></td>
<td>200 mg/mL</td>
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<td>1</td>
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Note: (-) bacterial growth appeared

**Table 1.** Data on Minimum Inhibitory Concentration showing no antibacterial effect on the extract concentration of 3.125-200 mg/mL.
Analysis of the Results

This study on antibacterial activity used extract ethanol of *Murraya paniculata* leaves dissolved in 40% ethanol. This study was conducted by comparing the antibacterial activity of extract ethanol of *Murraya paniculata* leaves with ethanol as the solvent in several concentrations, ranging from 200-3.125 mg/mL. MHB media were put in all tubes (except for the one with 200 mg/mL concentration) to reduce the extract concentration to half of the initial concentration.

After all tubes were filled with the required amount of concentration, the bacterial suspensions measured using McFarland’s 0.5 standard were added to each tube. Furthermore, the tubes were incubated in the temperature of 37°C for 24 hours. The results can be seen in tube 1 to 7 (the tubes appeared turbid, resembling the positive control tube). Similar thing occurred to the other tubes. Due to the turbidity, observation could not be carried out for MIC. Therefore, streaking was conducted on Nutrient Agar Plates to confirm the obtained results.

After incubated in 37°C temperature for 24 hours, there were bacterial colony growths in all Agar Plates. It was concluded that adding extract ethanol of *Murraya paniculata* leaves extract up to the concentration of 200 mg/mL is unable to inhibit the *Klebsiella pneumoniae* ESBL bacterial growth.

DISCUSSION

Dilution was carried out by preparing seven tubes containing MHB which then added with extract in certain concentrations and bacterial suspension. After incubated in the temperature of 37°C for 24 hours, the tubes were observed. Bacterial growth still occurred if the solution appeared more turbid than in the negative control. The extract was made from 100 grams of *Murraya paniculata* and 500 mL of 40% ethanol. This study was carried out four times according to Federer’s formula measurement. There were four mechanisms of bacterial resistance against β-lactam enzyme: β-lactam inactivation by β-lactamase enzyme; Penicillin Binding Protein (PBP) production with lower affinity against antibiotics; changes in porin channel leading to decreased permeability against antibiotics; and efflux pump that encourages antibiotics to escape the cells.12

*Klebsiella pneumoniae* ESBL is a bacterium that is able to produce β-lactamase enzyme, the enzyme that resist to all penicillin and cephalosporins, including the sulbactam and clavulanic acid combinations and monobactams such as aztreonam.13 The expression of β-lactamase enzyme induced by muropeptides, which is a product from cell wall metabolism of Gram-negative bacteria.14

According to the phytochemical assay, ethanol extract contains more secondary metabolite compounds than water extract does. Secondary metabolite compounds comprise alkaloids, flavonoids, saponins, triterpenoids, steroids, and tannins.15

Active ingredients of extract ethanol of *Murraya paniculata* leaves and are fathomed to have antibacterial effect are volatile oil, flavonoids, alkaloids, coumarins, terpenoids, saponins, and tannins. Volatile oil contains a compound acting as antibacterial by interrupting the forming of membranes or cell walls.16 Flavonoids, which is derived from phenol, show antibacterial activity since its penetration into cells causes protein precipitation, protein denaturation, protein coagulation, structure damage, and membrane lysis.17 Alkaloids interrupt peptidoglycan components in bacterial cells, causing cell walls not to form well and the cell itself to die.18 Coumarins show antibacterial activity due to its lipophilic structures and planar molecules that contribute to the penetration to cell
membrane or wall. Adding methyl or O-methyl group in the C6 or C7 position into coumarin aromatic core maintains the antibacterial activity in Gram-negative bacteria.25

Terpenoids as antimicrobial compounds whose mechanism of action is membrane disruption, could be futuristic biocide properties. It can be used in conjunction with other products such as antibiotics at sub-effective concentrations therefore it can confer bacterial resistance to antibiotics.19 Saponins extract of the A. articulate have antimicrobial activity on ranges of Gram-negative antibiotic-resistant isolates.20 Saponin compound in Acacia Arabica extract has antimicrobial activity against diarrheagenic E.coli.21 Saponin-rich extracts from guar meal and quillaja exhibited antibacterial activity against S.aureus.22 Tannins have phenolic group which can be as antimicrobial and formulation based on tannin-rich plants have been used as diarrhea treatment.23 The previous study proved antibacterial effect of Murraya paniculata extract. Ethanol extract in Murraya paniculata inhibits the growths of E.coli, K.pneumoniae, S.typhi, E.faecalis, P.aeruginosa, S.flexneri, S.aureus, and S.sonneii in 200 mg/mL concentration.24 Meanwhile, 200 mg/mL concentration of ethanol extract in Murraya paniculata non-significantly inhibits E.coli, P.mirabilis, S.Typhi and E.aerogenes.25

The results show that extract ethanol of Murraya paniculata leaves fail to inhibit and terminate Klebsiella pneumonia ESBL bacterial growth. This might be due to several matters, including: inhibition and termination of Klebsiella pneumonia ESBL require concentration of >200 mg/mL; combination with other antibiotics is required for optimum inhibition of Klebsiella pneumonia ESBL bacterial growth; further extraction is required until pure compound is obtained, enabling adjustment to optimum solvent.

In a study using Murraya paniculata ethanol extract with 300 mg/mL concentration, the growth of E.coli, P.mirabilis, S.typhi, dan E.aerogenes were inhibited significantly.25 Other study reported that total alkaloids extracted from Sophorea alpecuroides L. combined with cefotaxime or cefazidime against E. coli ESBL has MICs of 12.5 mg/mL.26 Total alkaloids increase bacterial susceptibility to cefotaxime and cefazidime by 8-16 times. Natural flavonoid combined separately with amoxicillin, clavulanic acid, ampicillin/sulbactam and cefoxitin synergically inhibit the activities of Klebsiella pneumoniae ESBL that is still susceptible to imipenem and cefmetazole.26

The three most studied coumarins include auraptene, umbelliprenin and 7-isopentoxyocoumarin.27 Auraptene inhibits bacterial activity producing β-lactamase class A.28

In conclusion, The six flavonoids: 5,7-dimethoxyflavanone-4′-O-β-D-glucopyranoside; 5,7-dimethoxyflavanone-4′-O-[2″-O-(5‴-trans-cinnamoyl)-6'-D-apiofuranosyl]-β-D-glucopyranoside; 5,7,3′-trihydroxy-flavanone-4′-O-β-D-glucopyranoside; naringenin 7-O-β-D-glucopyranoside; rutin; and nicotiflorin, inhibit the of Klebsiella pneumoniae ESBL growth.29

CONCLUSION

Extract ethanol of Murraya paniculata leaves with concentration 200 mg/mL shows no antibacterial effects against the growth of Klebsiella pneumoniae ESBL. The MIC of orange jessamine leaf extract is indeterminable.

ACKNOWLEDGEMENT

We thank you to Department of Microbiology Faculty of Medicine Universitas Airlangga.

REFERENCES


