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Antibacterial Activity of Leaf Extracts of *Anredera cordifolia* (Ten.) Steenis and *Muntingia calabura* L. Against *Streptococcus pneumoniae*

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ABSTRACT

Antibacterial resistance in Streptococcus pneumoniae has been increasing and is one of ongoing global concern. The need to find new antibacterial agents against Streptococcus pneumoniae is of paramount importance. Medicinal plants are prospective sources of antibacterial agents. The aims of the present study were to determine the activity of leaf extract of Anredera cordifolia (Ten.) Steenis and Muntingia calabura L. against Streptococcus pneumoniae. Leaves of Anredera cordifolia (Ten.) Steenis were extracted using 96% ethanol, while the leaves of Muntingia calabura L were extracted using 100% methanol. The leaf extracts of the two plants obtained were bioassayed for antibacterial activity against Streptococcus pneumoniae ATCC 49619 and a clinical isolate Streptococcus pneumoniae PU 067. Results showed that leaf extracts of both Anredera cordifolia (Ten.) Steenis and Muntingia calabura L. have antibacterial activity in vitro against Streptococcus pneumoniae ATCC 49619 at crude extract concentrations of 25%, 50%, 75% and 100% (w/v). Both plants extracts showed strongest activity against S. pneumoniae ATCC 49619 at extract concentration of 75%. In addition, the extracts of both plants have inhibitory activity against growth of the clinical isolate Streptococcus pneumoniae PU 067. Both plant extracts showed strongest activity against S. pneumoniae PU 067 at extract concentration of 100%. Therefore, leaf extracts of Anredera cordifolia (Ten.) Steenis and Muntingia calabura L. can potentially be used as a source of antibacterial agent for Streptococcus pneumoniae.

Keywords: Antibacterial agent, *Anredera cordifolia* (Ten.) Steenis, *Muntingia calabura* L., *Streptococcus pneumoniae*.

1. INTRODUCTION

Streptococcus pneumoniae was discovered in 1881, by two microbiologists independently, George M. Sternberg in the United States and Louis Pasteur in France. The bacterium was described as roughly lancet-shaped pairs of coccoid bacteria (Watson *et al.* 1993). *S. pneumoniae* is a Gram positive bacterium, the most common cause of pneumonia in the elderly. The bacterium also causes of middle ear infections and meningitis in children. Currently, vaccine for the pneumococcus is available consisting of a mixture of 23 different capsular polysaccharides. It is very effective in young adults, but only about 60% effective in the elderly and infected children under 2 years old. This is because children at this age group are unable to mount an antibody response to the pneumococcal polysaccharides. Antibiotics such as penicillin have played important roles in diminishing the risk from pneumococcal disease. Several pneumococcal proteins including pneumococcal surface proteins A and C, hyaluronate lyase, pneumolysin, autolysin, pneumococcal surface antigen A, choline binding protein A, and two neuraminidase enzymes are being investigated as potential vaccine or drug targets (Jedrzejewski 2001). However, the emergence of drug-resistant *S. pneumoniae*, especially multidrug-resistant (MDR) strains, decreases the effectiveness of antibiotics.

Multidrug-resistant *S. pneumoniae* has spread worldwide. In addition, the spread of extensively drug-resistant (XDR) *S. pneumoniae* has also been reported (Cho *et al.* 2014). Increased antimicrobial resistance in *S. pneumoniae* has been a worldwide concern mainly due to increase

used of antibacterial agents (Golden *et al.* 2015). The excessive use of antibacterial agents causes selective pressure that maintains strains harboring resistance genes once introduced into communities. It is well known that pneumococci have the ability to acquire resistance genes through natural transformation, even from other bacterial species (Vilhelmsson *et al.* 2000). Globally, *S. pneumoniae* causes ~582000 to 926000 deaths annually and antibacterial agents have been the first option for treating these infections. Therefore, it is critical to search for new effective antibacterial agents against *S. pneumoniae*.

Plant extracts have been investigated for use as a source of novel antibacterial agents (Iauk *et al.*, 2003; Njimoh *et al.*, 2015; Rahmawati *et al.*, 2017). *A. cordifolia* (Ten.) Steenis, commonly known as “binahong” in Indonesia, is one of the medicinal plants that has been shown to have pharmacological activities. In addition to its antibacterial activities, binahong has been reported to have antidiabetic, antiobesity, antihyperlipidemic, vasodilator and wound healing activities (Leliqia *et al.* 2017). Previous studies have shown that ethanolic extract of *A. cordifolia* leaves showed antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, methicillin-resistant coagulase-negative staphylococci (MRCNS), and *Pseudomonas aeruginosa* (Garmana *et al.* 2014). Other studies have also shown that an ethanolic extract, n-hexane and ethyl acetate fractions of *A. cordifolia* leaves have antibacterial activities against *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *Bacillus subtilis* and *Bacillus cereus* (Leliqia *et al.* 2017). Similarly,

Muntingia calabura L., commonly known as “kersen” in Indonesia, has been shown to have antibacterial activities and other pharmacological potential such as cytotoxic, antiproliferative, insecticidal, antioxidant, anti-inflammatory, hepatoprotective, hypotensive, antinociceptive, cardioprotective, antipyretic, antiplatelet aggregation, antiulcer and antidiabetic potential (Mahmood *et al.* 2014; Zakaria *et al.* 2019). The ethyl acetate partition of *Muntingia calabura* L. leaves methanol extracts were found to effectively inhibit growth of *Staphylococcus aureus* 25923 and *S. aureus* 33591 (a multi-drug resistant *S. aureus*, MRSA) (Zakaria *et al.* 2010). The methanol extracts of *Muntingia calabura* L. leaves and of *Muntingia calabura* L. fruits were also found to inhibit growth of *Escherichia coli* (C600) and *S. aureus* (209 P) (Mahmood *et al.* 2014). The present study was aimed to test the antibacterial activities of leaf extracts of *A. cordifolia* (Ten.) Steenis and *Muntingia calabura* L. against *S. pneumoniae*. To the best of our knowledge, this is the first study exploring the antibacterial activity of *A. cordifolia* (Ten.) Steenis and *Muntingia calabura* L. leaf extracts against *S. pneumoniae*.

2. METHODOLOGY

Preparation of Samples (Rahmi *et al.* 2014)

Samples in the form of *A. cordifolia* (Ten.) Steenis leaves were collected from Bogor, West Java, Indonesia. Leaves were cleaned and washed under running water and then air-dried at room temperature protected from the sun. The samples were

then dried in the oven at 50°C. The dried samples were then ground and extracted.

Extraction of samples (Rahmi *et al.* 2014; Zakaria *et al.* 2019)

Samples were extracted using a maceration technique. *A. cordifolia* (Ten.) Steenis leaf samples were extracted using 96 percent ethanol. The sample preparation powder was soaked in the extracting solvent with sample:solvent ratio of 1:10 (w/v) for 24 h. The sample preparation powder of *Muntingia calabura* L. leaf was extracted using 100% methanol with sample:solvent ratio of 1:4 (w/v) for 24 h. Both extracts were then concentrated using rotary vacuum evaporator. Solvent free-extracts of *A. cordifolia* (Ten.) Steenis and *Muntingia calabura* L. were obtained, and then dissolved in ddH₂O. Extract with concentration of 25%, 50%, 75%, and 100% (w/v) was obtained by dissolving 0.025 g, 0.050 g, 0.075 g, and 0.100 g of each extract in 100 mL ddH₂O, respectively.

Preparation of bacterial cultures (Safari *et al.* 2014)

Bacterial strains, *S. pneumoniae* ATCC 49619 and a clinical isolate of *S. pneumoniae*, PU 067, which were then used for the extract susceptibility test, were firstly activated by growing them in blood-agar medium under anaerobic conditions at 37°C, CO₂ level less than 5%, for 24 h. The bacterial growth was then observed.

Extract Susceptibility Testing

Extract susceptibility test for the pneumococcus isolates was carried out using the disk diffusion method according to Bauer *et al.* (1966). Following

incubation for 24 h, the testing bacteria were inoculated into an Eppendorf tube containing 2 mL of NaCl. The bacterial suspension was vortexed and its turbidity was then compared to the McFarland 0.5 turbidity standard. Using sterile cotton buds, the bacterial suspension was inoculated onto a blood-agar plate. An extract-containing 6 mm disk was then applied onto the bacterial culture and the plate was incubated at 37°C for 24 h under anaerobic conditions. The antibacterial activity was assayed by measuring the inhibition zone around the disk.

3. RESULT

Antibacterial activity of extract of *Anredera cordifolia* (Ten.) Steenis

Extract of *A. cordifolia* (Ten.) Steenis was obtained in the form of a paste. The yield of the extract was 9.53%. The extract was then dissolved in ddH₂O and used for antibacterial activity test. Results of antibacterial activity test of the extract of *A. cordifolia* (Ten.) Steenis at various concentrations are shown in Table 1. The leaf extracts of *A. cordifolia* (Ten.) Steenis inhibited the growth of *S. pneumoniae* ATCC 49619 at crude extract

Table 1. Antibacterial activity of leaf extract of *Anredera cordifolia* (Ten.) Steenis

<i>Anredera cordifolia</i> leaf extract concentration (%)	Diameter of inhibition zone (mm)	
	<i>S. pneumoniae</i> ATCC 49619	<i>S. pneumoniae</i> PU 067
25	10.9	8.0
50	11.4	9.6
75	14.0	10.9
100	10.2	14.0
ddH ₂ O (negative control)	6.0	6.0
Chloramphenicol (30 µg)	29.7	29.4

Table 2. Antibacterial activity of leaf extract of of *Muntingia calabura* L.

<i>Anredera cordifolia</i> leaf extract concentration (%)	Diameter of inhibition zone (mm)	
	<i>S. pneumoniae</i> ATCC 49619	<i>S. pneumoniae</i> PU 067
25	13.0	9.0
50	13.0	13.0
75	13.3	13.4
100	10.8	16.3
ddH ₂ O (negative control)	6.0	6.0
Chloramphenicol (30 µg)	29.7	29.4

concentrations of 25%, 50%, 75% and 100% (w/v). Similarly, the extracts inhibited the growth of *S. pneumonia* PU 067 at crude extract concentrations of 25%, 50%, 75% and 100% (w/v). The optimum extract concentration to inhibit *S. pneumoniae* ATCC 49619 and *S. pneumonia* PU 067 was 75% and 100%, respectively. The inhibitory activity of the extract at all concentrations tested was lower than that of chloramphenicol (30 µg) against the two strains.

Antibacterial activity of leaf extract of *Muntingia calabura* L.

Extract of *Muntingia calabura* L. was also obtained in the form of a paste with a yield of 9.54%. The extract was then dissolved in ddH₂O and used for antibacterial activity test. Results of antibacterial activity test at various extract concentrations are shown in Table 2. The leaf extracts of *Muntingia calabura* L inhibited the growth of *Streptococcus pneumoniae* ATCC 49619 at crude extract concentrations of 25%, 50%, 75% and 100% (w/v). Similarly, the extracts inhibited the growth of resistant *Streptococcus pneumonia* isolate at crude extract concentrations of 25%, 50%, 75% and 100% (w/v). The optimum *Muntingia calabura* L extract concentration to inhibit *S. pneumoniae* ATCC 49619 and *S. pneumonia* PU 067 was 75% and 100%, respectively. The inhibitory activity of the *Muntingia calabura* L extract at all concentrations tested was also found to be lower than that of chloramphenicol (30 µg) against the two strains.

4. DISCUSSION

We report experimental data of antibacterial activity of leaf extracts of binahong (*Anredera cordifolia* (Ten.) Steenis) and kersen (*Muntingia calabura* L.) at various concentrations against *S. pneumoniae* in attempt to search for novel sources of antibacterial agents against *S. pneumoniae* including the MDR and XDR strains. This is important as resistant strains of *S. pneumoniae* have been reported to be present in Indonesia (Safari *et al.*, 2014). Two *S. pneumoniae* strains were used in this study, the *S. pneumoniae* ATCC 49619 and a multidrug resistant clinical isolate, *S. pneumoniae* PU 067. The strain *S. pneumoniae* PU 067 is resistant towards penicillin group antibiotics, oxacillin, sulfamethoxazole/trimethoprim, and tetracycline (Saputro 2013). In the present study, the extracting solvents used were ethanol 96% to produce leaf extract of *Anredera cordifolia* (Ten.) Steenis and methanol 100% to produce leaf extract of *Muntingia calabura* L. For antibacterial assays, ddH₂O was used as a negative control and chloramphenicol (30 µg) was employed as a positive control. Our results showed that the leaf extracts of binahong and kersen have inhibitory activity against both *S. pneumoniae* ATCC 49619 and *S. pneumoniae* PU 067. The activity, however, is lower than the activity of the antibiotic chloramphenicol. The inhibitory activity against *S. pneumonia* PU 067 tended to increase with the increase concentration of both *Anredera cordifolia* (Ten.) Steenis and *Muntingia calabura* L extracts. Compared to the *Anredera cordifolia* (Ten.) Steenis extract, the

Muntingia calabura L extract seemed to be slightly more potent in inhibiting the growth of the multidrug resistant *S. pneumoniae* PU 067. Interestingly, both plants extracts showed a higher optimum concentration to inhibit the *S. pneumoniae* PU 067. The reasons for this have yet to be elucidated but may be associated with the resistant characteristics of the *S. pneumoniae* PU 067 strain. Further studies, therefore, are required to elucidate the compounds responsible for the antibacterial activity and the molecular mechanism underlying the *S. pneumoniae* growth inhibitory activity.

In Indonesia, the availability of data on the spread and disease level of *S. pneumoniae* are limited among the Indonesian population. It was reported that *S. pneumoniae* spread in Lombok Island in 2001 was 48% in healthy children. Similarly, *S. pneumoniae* spread in Semarang in 2010 was reported to be 43% in children aged 6-60 months and 11% in adults aged 45-75. In HIV-infected group of children in Jakarta the *S. pneumoniae* it was 46%, and 3% in elderly age 60-80 years attending routine visits at the Geriatric Clinic, Dr Cipto Mangunkusumo Hospital, Jakarta (Safari et al. 2015). The serotypes of *S. pneumoniae* identified from the HIV infected children in Jakarta include serotype 19F, 19A, 6A/B and 23F. Most of the isolates were susceptible to chloramphenicol, clindamycin, and erythromycin, but resistant to penicillin (Safari et al. 2014). Globally, there has been a dramatic increase in the incidence of penicillin-resistant and multiply-antibiotic-resistant pneumococci

worldwide since 1967 (Charpentier and Toumanen 2000). The mechanism of penicillin resistance in *S. pneumoniae* has been suggested to involve the alteration of Penicillin-binding proteins (PBPs) which leads to reduced affinity of the PBPs to the antibiotic molecule. Mutations leading to penicillin resistance usually occur in the transpeptidase-penicillin-binding domain. Multiple mutations are required for high-level resistance to occur. In pneumococcus, five PBPs of high molecular weight (PBPs 1a, 1b, 2x, 2a and 2b) and one PBP of low molecular weight have been reported. Mutations in PBP2x and PBP2b bring about low-level resistance and are the prerequisite for high-level resistance mediated by mutations in other PBPs, like PBP1a. In many pneumococcal clinical isolates resistance is caused by changes in these three PBPs only (Charpentier and Toumanen 2000).

Binahong is a medicinal plant belonging to the family of Basellaceae, and is considered to have high potency for phytopharmaceutical sources. Binahong has been reported to contain chemical constituents such as flavonoid, oleanolic acid, protein, saponins, steroids, terpenoids, phenols, polyphenols, alkaloids tannin, ascorbic acid, and mono polysaccharides including L-arabinose, D-galaktose, and L-rhamnose (Astuti 2011; Wijayanti and Esti 2017; Yuniarti and Lukiswanto 2017). The antibacterial activity of binahong leaf has been reported (Miladiyah and Prabowo 2012; Yuniarti and Lukiswanto 2017). The bioactive compounds of binahong have been indicated to play roles in its antibacterial activities against both Gram-

positive and Gram-negative bacteria including those causing sexually transmitted diseases (Astuti 2011). Kersen is a plant introduced from Tropical America to Southeast Asia and has traditional medicinal usage in various modes of applications. Kersen have been suggested to have anti-inflammatory, anti-nociceptive antioxidant and antimicrobial activities as well as cytotoxicity against leukemia cell lines. The ethanolic leaf extracts of *M. calabura* were reported to contain sterols, flavonoids, alkaloids, saponins, glycosides, and tannins. Triterpenes were not detected in the leaf extracts but detected in the stem extracts of the plant. The *M. calabura* extracts were found to have antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The polyphenols, especially the flavonoids have been linked to the antibacterial activities (Buhian *et al.* 2016).

Extraction is a critical step in the evaluation of antibacterial activity medicinal plants. It is important to ensure that the chemical components having antibacterial activities are extracted from the plant materials. The basic steps in preparing plant extract include pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Care must be taken to prevent loss of potential active constituents. If the plants are selected on the basis of traditional uses it is necessary to prepare the extract as described by the traditional healer in order to mimic as

closely as possible the traditional 'herbal' drug. Extraction methods using polar solvents such as methanol, ethanol or ethyl-acetate are generally used to extract hydrophilic compounds. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol can be used (Sasidharan *et al.* 2011). In the present study, binahong leaf was extracted by maceration using 96% ethanol and the kersen leaf was extracted by the same technique using methanol 100%. The extracts showed antibacterial activity against *S. pneumoniae* ATCC 49619 and a clinical isolate of MDR *S. pneumoniae*. Future studies employing a more systematic and comprehensive approach is required to confirm the potency of *A. cordifolia* (Ten.) Steenis) and *M. calabura* L. leaves as a source of novel antipneumococcal agents. Modern extraction techniques such as solid-phase micro-extraction, supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, solid-phase extraction, and surfactant-mediated techniques, possess certain advantages (Sasidharan *et al.* 2011) and may be applicable for extracting compounds with antibacterial activities from *A. cordifolia* (Ten.) Steenis and *M. calabura* L.

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