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Efficacy of Ethanol Extract of Sambiloto (*Andrographis paniculata*) Against Pneumonia-Causing *Klebsiella pneumoniae*

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ABSTRACT

Klebsiella pneumoniae is one of the most common pathogens in nosocomial respiratory infections. Antibiotics are often used for the treatment of *K. pneumoniae* infections. Increased bacterial resistance to antibiotics is closely related to the success rate of infectious disease therapy. The use of plants as phytopharmacology is currently an alternative treatment. Some medicinal plants that have antibacterial and immunomodulatory activity are Sambiloto (*Andrographis paniculata*) with an active compound called andrographol. The purpose of this study was to provide a solution to the problem above by developing a phytopharmaceutical using bitter extract to inhibit the growth of *K. pneumoniae* bacteria. Two sputum samples from the laboratory confirmed Covid-19 patients with pneumonia infection at the Hospital. The sambiloto was extracted by using 96% ethanol. The extract was further analyzed for its antimicrobial activity and phytochemical compounds. There were two *K. pneumoniae* isolates, namely KpRNG and KpTRI. The active compounds were saponin and steroids. The antimicrobial activity of the ginger extract was measured with different concentrations of 25%, 50%, 75%, and 100%. Its inhibitory zone against *K. pneumoniae* was ranging from 5.8 to 7 mm or had a moderate inhibition category.

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INTRODUCTION

Nosocomial infections are a big challenge worldwide. *Klebsiella pneumoniae* is one of the most common pathogens in community-acquired and nosocomial respiratory infections. The high spread of *K. pneumoniae* bacteria in the hospital environment causes nosocomial infections (Harapan *et al.*, 2018). In recent years, nosocomial infection with *K. pneumoniae* resulted in high morbidity and mortality (Huang *et al*., 2022). *K. pneumoniae* can be found in the respiratory tract and intestines of healthy people and animals. However, these bacteria can cause opportunistic infections in various tissues and organs of the body, meningitis, liver abscesses, urinary tract infections, and sepsis, and cause pneumonia (Huang *et al*., 2022; Zhang *et al*., 2019).

The Ministry of Health reported that pneumonia is one of the acute respiratory infections (ARI), a notable problem in developing countries, including Indonesia. Globally, pneumonia due to infection with K. pneumoniae is characterized by an exacerbation of the immune response, intense lung injury, coughing (bronchitis), thickening of the mucosal walls, bloody sputum, and even death (Chansg *et al*., 2013). K. pneumoniae infection is responsible for a mortality rate of up 50% in the elderly (Patel Singh *et al*., 2021). This bacterial infection in infants, the elderly, and immunocompromised patients, is often life-threatening (Anand *et al*., 2020), and often complicated by bacteremia and sepsis (Stahlhut *et al*., 2012).

Antibiotics are often used for the treatment of *K. pneumoniae* infections. Carbapenems are widely used as the drug of choice for severe infections caused by *K. pneumoniae*, especially strains that produce beta-lactamases (Shaikh *et al*., 2015). However, the efficacy of current treatment approaches has decreased, as these bacteria have increased their resistance to various antibiotics. *K. pneumoniae* carries a variety of antimicrobial resistance genes, including extended-spectrum b-lactamases (ESBLs) and carbapenemases (Huang *et al*., 2022). *K. pneumoniae* which produces extended-spectrum beta-lactamase and is carbapenems-resistant introduces significant challenges to the use of antibiotics. In 2013, the prevalence of *K. pneumoniae* ESBL cases reached 37% in America and 43-55% in Denmark (Harapan *et al*, 2018).

Increased bacterial resistance to antibiotics is closely related to the success rate of infectious disease therapy. Bacterial resistance can lead to failure in response to treatment, including prolonging the period of illness, prolonging the period of hospitalization, increasing the burden of financing, and even increasing the risk of death (Harapan *et al*, 2018). Therefore, alternative antibacterial material is needed that can inhibit the growth of *K. pneumoniae*. The use of plants as phytopharmacology is currently an alternative treatment.

Various plants have long been used as traditional medicine by people in Indonesia. The use of plants as medicinal ingredients is considered more affordable for the community compared to the cost of buying drugs or antibiotics. Sambiloto is one of the plants used as a medicine for infectious diseases. Sambiloto (*Andrographis paniculata*) contains several active compounds, such as andrographolide. This compound can act as an immunomodulator which improves the work of the immune system (Priyani, 2020). Previous research was conducted by Suryelita *et al*., 2021 that bitter Andrografis paniculate showed antibacterial activity against three (3) bacteria tested; *E. coli*, *S. aureus* and *S. pyogenes*.

Traditional medicine or phytopharmacology requires the support of scientific research, such as toxicology and pharmacology research. This study aimed to explore the potential of sambiloto as an antimicrobial, especially *K. pneumoniae* from Covid-19 patients. This study is expected to provide a scientific basis for the use of sambiloto in infectious disease treatment.

MATERIALS AND METHODS

Sample source

Sputum specimen samples were isolated from patients with confirmed Covid-19 pneumonia diagnosis treatment at Cilacap Hospital.

Sambiloto extraction

Sambiloto extraction is carried out by using the simplest method. Sambiloto leaves are washed, cleaned, and dried in an oven for 2 days at a temperature of 80-100℃. The dried sambiloto leaves are crushed by pounding and then weighed. Simplicia (the result of pounding leaves) as much as 500 grams was dissolved and stirred with 96% ethanol solvent extraction material with a volume of 500 ml for 3x24 hours. The mixture is then filtered using a vacuum filter every 1x24 hours, then the sample is extracted using a rotary evaporator (Utami *et al*, 2020).

Sambiloto liquid extract formulation with various concentrations
Sambiloto extract was made in 4 different concentrations, namely 100% (S4), 75% (S3), 50% (S2), and 25% (S1). The 100% test solution was obtained by adding 10 ml of sambiloto extract. The 75% concentration test solution was obtained by adding 7.5 ml extract and 2.5 ml sterile distilled water. The 50% concentration test solution was obtained by adding 5 ml extract and 5 ml sterile distilled water. The 25% concentration test solution was obtained by adding 2.5 ml extract and 7.5 ml aquadest.

Preparation of *K. pneumoniae* culture
K. pneumoniae isolates were cultured by streaking on cysteine-lactose-electrolyte-deficient (CLED) agar, then incubated at 37°C for 24 hours. The growing bacterial colonies were purified and characterized according to colony morphology. Identification of bacteria based on physiological and biochemical tests using the bioMeriuex Vitek 2 compact system version 07.01.

Bacterial sensitivity test *K. pneumoniae*
K. pneumoniae isolates were transferred to a physiological NaCl 0,45% solution. The bacterial isolate used had a turbidity standard of 0.5 Mc Farland. The liquid isolate of *K. pneumoniae* was rubbed on the surface of CLED agar. Disc paper was dipped in each test solution for 30 seconds. The paper disc is placed on top of the CLED agar media. The positive control used was the antibiotic ampicillin 25 g. Sterile aquadest was used as a negative control. Bacterial growth media were incubated at 37°C for 24 hours. The clear zone that appears around the paper disc is observed and its diameter is measured. The inhibition zone obtained is the result of the average measurement with 3 replications. The research method used was a Randomized Block Design (RBD) which consisted of 3 treatment groups with 4 differences and 3 replications. Negative Control: sterile distilled water. Positive Control: *K. pneumoniae* isolate + antibiotic ampicillin. Treatment: *K. pneumoniae* isolate + sambiloto extract (100% (S4), 75% (S3), 50% (S2), and 25% (S1)).

Data analysis
Data on sambiloto extract between concentration of bacterial sensitivity were analyzed using ANOVA. After checking for normality, means were separate with Tukey test at the 5% significant level.

RESULTS AND DISCUSSION

Pneumonia becomes a significant killer of children's diseases globally. WHO reported that 15% of deaths in children under five years were caused by pneumonia in 2017 (www.who.int). UNICEF states that more than 1400 cases per 100,000 children are reported to have pneumonia every year. In Indonesia, the prevalence rate of pneumonia in children under five is relatively high, namely 4.5 per 100 children under five (Basic Health Research, 2013). In 2016, the number of pneumonia sufferers was more than 800,000 children (Basic Health Research, 2018). To prevent deaths from pneumonia, the Indonesian government has encouraged the Integrated Management of Childhood Illness (IMCI). IMCI is carried out passively at the Puskesmas. The government hopes that pneumonia diagnosis is carried out early at the age of toddlers, improving management, health promotion, and increasing maternal knowledge.

Resistance to *Klebsiella pneumoniae* which causes pneumonia continues to increase. To address this condition, researchers explore the potential of sambiloto as medicine for infectious diseases. In this study, samples were taken from patients with coronavirus disease (Covid-19) with symptoms of pneumonia. There were two isolates of *K. pneumoniae* obtained, namely KpRNG and KpTRI isolates. A high prevalence (48.6%) of ESBL-producing *K. pneumoniae* is recorded in Covid-19 patients in Indonesia. It is commonly found in the elderly (41.2%) and contributes to the severity of Covid-19 infection (Ahmad *et al*., 2021).

Andrographis paniculate is very well known in Indonesia as a medicinal plant because of its sambiloto taste. It is known locally as sambiloto, traditionally used to treat various diseases such as high blood pressure, fever, malaria, diabetes, gastrointestinal disorders, inflammation, dysentery, and cancer (Sholikhah, 2016). The sensitivity test showed that sambiloto extract could inhibit the growth of clinical isolates of *Klebsiella pneumoniae* KpRNG and KpTRI. Sambiloto extract at a concentration of 25-100% produced an inhibition zone with a diameter ranging from 5.8-7 mm or had a moderate inhibition category (Table 1). *K. pneumoniae* is a Gram-negative bacteria and has a faster resistance rate than Gram-positive bacteria. It is because *K. pneumoniae* produces betalactamase enzymes (Sah *et al*., 2020).

Table 1. The sensitivity of *K. pneumoniae* isolates KpRNG and KpTRI to sambiloto extract at a concentration of 25-100%.

Code	Consentration	Diemeter of the inhibitory zone (mm)			Average	Category
		1	2	3		
KpRNG-S1	25%	6	6	6	6	moderate
KpRNG-S2	50%	6	7	6	6,3	moderate
KpRNG-S3	75%	7	7	7	7	moderate
KpRNG-S4	100%	7	7	7	7	moderate
KpTRI-S1	25%	5,5	6	6	5,8	moderate
KpTRI-S2	50%	6	6	6	6	moderate
KpTRI-S3	75%	6	6	7	6,3	moderate
KpTRI-S4	100%	7	6	6	6,6	moderate
N (S0)	Negative control	0	0	0	0	-
P (SP)	Positive control	20	20	20	20	very strong

remarks: antimicrobial category based on the diameter of the formed inhibition zone (mm). 20 mm: very strong, 10-20 mm: strong, 5-10 mm: moderate, 5 mm: weak (Silaban, 2021).

The results of observations that have been carried out with negative control (S0) images do not show an inhibition zone, and in positive controls with strong inhibition reaching a zone area of 20 mm, compared to the test results on the KpRNG-S3 isolate inhibited at a concentration of 75% (S3) sambiloto treatment with an average inhibition of 7 mm in the moderate category, and the KpRNG-S4 isolate was optimally inhibited at a concentration of 100% (S4) with an average inhibition zone of 7 mm. Other isolates showed differences in inhibition zones in the KPTRI-S3 isolate with a concentration of 75% (S3), the average inhibition zone was 6.3 mm, while in the KpTRI-S4 isolate with a concentration of 100% (S4), the inhibition zone reached 6.6 mm, in the moderate category. This inhibition was very different with a p-value of 0.012-0.0825 from the inhibition of the positive control (ampicillin) which was 20 mm (very strong). The results of ANOVA showed that the KpRNG isolates treated with sambiloto extract with different concentrations resulted in the conclusion that H0 was accepted, which means that between treatments with a concentration of 25-100% there was no significant difference S0<S1<S2<S3<S4, S0 negative control. The effective sambiloto extract used as an antibacterial was 100% concentration with a p-value of 0.012-0.0825. The treatment of giving sambiloto extract, illustrates that there is no significant difference between each treatment, namely 25%, 50%, 75%, and 100%, but has a positive inhibitory effect when compared to the negative control value which has no inhibition. Giving the extract sambiloto provides optimal inhibition of the growth of *K. pneumoniae* reaching 7 mm in the moderate category, this is in accordance with (Suryelita *et al*., 2021) that sambiloto extract can inhibit the growth of *E. coli*, *S. aureus*, and *S. pyogenes*.

The results of the study produced phytochemical analysis tests on the sambiloto extract obtained steroid and saponin components (Table 2). Steroids are marked by the formation of a reddish-brown ring. The formation of saponins is marked by the formation of an emulsion. Steroids might be the most abundant phytochemical present. Phytochemical testing on sambiloto leaf extract identified it as containing flavonoids, alkaloids, saponins, and tannins (Komori *et al*., 2020). In inhibiting bacterial growth, saponins play a role in destroying the cytoplasmic membrane of bacteria which can result in reduced cell membrane permeability resulting in uncontrolled transportation of substances from within and out of cells (Brigitta *et al.,* 2021). It affects organic ions such as enzymes, amino acids, and nutrients out of the cells, thereby inhibiting bacterial metabolism by decreasing ATP causing bacterial cell death. Other active compounds, namely flavonoids and tannins, in their mechanism of action, flavonoids also damage the permeability of the walls of bacteria, microsomes, and lysosomes, besides that alkaloid compounds can disrupt the peptidoglycan composition of the bacterial cell wall causing the cell wall to not form completely and result in cell death (Silaban, 2021). Gram-negative strains were less sensitive to plant extracts than Gram-positive ones. The outer membrane of the cell wall of Gram-negative bacteria appears to act as a barrier to many substances, including antibiotics (Sule *et al*., 2010).

Table 2**.** Initial phytochemical analysis of the sambiloto extract

Phytochemical constituents	Phytochemical characteristics	
	code	description
Tanin	-	The green precipitate is not formed
Flavonoid	-	Yellow color is not formed
Terpenoid	-	The dark red color is not formed
Saponin	+	Emulsion formed
Steroid	++	A reddish-brown ring is formed
Alkaloid	-	A yellow precipitate is not formed

remarks: + = Positive compound content (exists); ++= Positive compound content (exists, the value is higher)

A. paniculata showed effective inhibitory zone formations against both Gram-negative and Gram-positive bacteria, including *Aeromonas hydrophila*, *Bacillus subtilis*, *Escherichia coli*, *K. pneumoniae*, *Micrococcus luteus*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhi* (Doss & Kalaichelvan, 2012; Nayak *et al*, 2015; Sah *et al*., 2020). Nayak *et al*, (2015) investigated the antibacterial potential of *A. paniculata* against Gram-positive and Gram-negative bacteria. Nur Rachmani *et al*, (2018) found that sambiloto herbs contain the active compound ethyl acetate with andrographolide and flavonoids. This compound has antioxidant activity with a strong category, namely 3.43 g/mL. Andrographolide is the main bioactive compound presented in *A. paniculata* (Nayak *et al*, 2015). Phytocompounds in the methanol extract of *A. paniculata*, especially 3-O-b-D- glucosyl-14 deoxyandrographolide and 14-deoxyandrogropholide exhibited antibacterial efficiency (Sah *et al*, 2020). Clinically, andrographolide compounds can increase the work of the immune system in patients with immune disorders, including; cases of cancer, HIV/AIDS, malnutrition, and allergies (Alkandahri *et al*., 2018).

The use of sambiloto as an antibacterial extract material might depend on the quality of the produced extract. Several factors affect the quality of the extract. Biological and chemical conditions of plants and good extracts can produce quality extracts. The type of sambiloto plant is biologically influential, in addition to the location of origin of the plant, time of harvest, age of the plant, storage of raw materials, and the parts used in the manufacture of extracts (leaves; stems; roots) (Hardodianto *et al*., 2000). This study used sambiloto leaf as a source of extract. The quality of the extract is also influenced by chemical factors, namely internal and external. The internal factors include the type, composition, and content of compounds in the extract. The external factors dependent on the extraction method used, include; size, filter, heavy metal content, and pesticides.

CONCLUSIONS AND SUGGESTION

The ethanol extract of sambiloto (*A. paniculata*) demonstrates promising antibacterial activity against *K. pneumoniae*, a clinical isolate known to cause pneumonia in Covid-19 patients. With measurable inhibition at concentrations ranging from 25% to 100%, sambiloto extract shows potential as a natural antimicrobial agent, achieving inhibition zones averaging 5.8-7 mm. However, further research, including in vivo testing and clinical trials, is essential to develop a standardized, safe, and effective herbal formulation of sambiloto. This future work will be critical to validating sambiloto's therapeutic potential and ensuring its safe application in clinical settings.

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