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ORIGINAL ARTICLE

Antibacterial activity of ashitaba leaf extract against *Streptococcus pneumoniae*

Tommy Daniel Patar P. Hutabarat[1], Suandy[2,3]*, Oliviti Natali[2,3]

ABSTRACT

Infection by *Streptococcus pneumoniae* represents a significant global health concern, necessitating the exploration of novel antimicrobial agents, particularly in light of escalating antibiotic resistance. This study aimed to identify the phytochemical constituents and to evaluate the antibacterial activity of an ethanol extract of Ashitaba leaves (*Angelica keiskei*) against *S. pneumoniae*. This experimental study employed a post-only control group design. Extraction was performed using ethanol via maceration, followed by qualitative phytochemical screening. Antibacterial activity was assessed using the disc diffusion method at concentrations of 50, 100, and 150 mg/mL, with ciprofloxacin as a positive control. Inhibition zone data were analysed using non-parametric statistical tests. Phytochemical screening indicated the presence of flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids. The extract demonstrated antibacterial activity with mean inhibition zones of 16.57 mm at 50 mg/mL, 26.23 mm at 100 mg/mL, and 29.87 mm at 150 mg/mL. Statistical analysis revealed no significant difference in inhibition zones among the three extract concentrations, whereas all concentrations differed significantly from the positive control, ciprofloxacin (53.67 mm). It is concluded that the ethanol extract of Ashitaba leaves contains diverse bioactive compounds and exhibits significant *in vitro* antibacterial activity against *S. pneumoniae*, although its efficacy remains inferior to the synthetic antibiotic ciprofloxacin.

Keywords: ashitaba, antibacterial, ethanol extract, *Streptococcus pneumoniae*

Introduction

Pneumonia is an acute infection of the pulmonary parenchyma caused by various pathogens.[1] Lower respiratory tract infections remain a leading cause of mortality from infectious diseases, accounting for an estimated 3 million deaths globally in 2016.[2] Among children under five years of age, pneumonia is the single highest cause of death, responsible for 15% of all fatalities in this age group.[3] In Indonesia, the prevalence of pneumonia diagnosed by healthcare professionals increased from 1.6% in 2013 to 2.0% in 2018.[4] A similar increasing trend was observed in North Sumatra, where prevalence based on healthcare diagnosis rose from approximately 1% to 2.25% during the same period.[5] *Streptococcus pneumoniae* is a principal bacterial pathogen responsible for community-acquired pneumonia (CAP).[6] The rising prevalence of antibiotic-resistant *S. pneumoniae* strains poses a significant challenge in managing these infections, underscoring the need to explore novel antimicrobial agents from alternative sources, such as medicinal plants.[7]

Affiliation
[1]Undergraduate Program in Medical Science, Universitas Prima Indonesia
[2]Department of Biomedicine, Universitas Prima Indonesia
[3]PUI Phyto Degenerative & Lifestyle Medicine, Universitas Prima Indonesia

*Corespondence:
suandy76@gmail.com

The Ashitaba plant (*Angelica keiskei* Koidzumi) represents a potential source of natural antimicrobial agents. Traditionally used in ethnomedicine, it has been reported to possess various pharmacological properties, including antidiabetic, antioxidant, anti-inflammatory, and antimicrobial effects.[8] These biological activities are attributed to its diverse bioactive constituents, primarily flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids.[9] Previous studies have demonstrated the antibacterial activity of Ashitaba extracts against Gram-positive bacteria such as *Staphylococcus epidermidis*[10] and *Streptococcus mutans*.[11] However, data on the efficacy of Ashitaba extract specifically against *S. pneumoniae* remain limited and warrant further investigation. Given the urgent need for alternative antimicrobials and the potential of Ashitaba leaves, this study was conducted to identify the phytochemical constituents of an ethanol extract of Ashitaba leaves (*Angelica keiskei*) and to test its antibacterial activity against *Streptococcus pneumoniae in vitro*.

Method

This laboratory-based experimental study utilised a post-only control group design. The research was conducted at the Integrated Laboratory of Universitas Prima Indonesia from July to September 2025. The independent variable was the concentration of the Ashitaba leaf ethanol extract (50, 100, and 150 mg/mL), while the dependent variable was the diameter of the inhibition zone against *S. pneumoniae* growth, measured in millimetres (mm). Ashitaba leaf samples were obtained from a traditional medicine store in Medan City and were botanically identified at the Medanense Herbarium, FMIPA Universitas Sumatera Utara. The test microorganism used was a pure culture of *Streptococcus pneumoniae*.

The research procedure comprised several stages. First, simplicia preparation and extraction. Ashitaba leaves were washed, dried using a food dehydrator, and ground into a powder. A total of 500 g of powder was macerated with 96% ethanol (1:10 ratio) for three days with periodic agitation. The filtrate was collected and concentrated using a rotary evaporator at 40-50°C to obtain a viscous extract. The extraction yield was calculated based on the initial powder weight. Second, qualitative phytochemical screening. The viscous extract was tested for secondary metabolite groups, including flavonoids (reaction with Mg and concentrated HCl), alkaloids (Dragendorff's reagent), saponins (foam test), tannins (reaction with 1% $FeCl_3$), glycosides (Molisch's test), and steroids/triterpenoids (Liebermann-Burchard test) according to standard methods.[12],[13] Third, preparation of test extract concentrations. The viscous extract was dissolved in dimethyl sulfoxide (DMSO) to create a 500 mg/mL stock solution, which was then serially diluted to concentrations of 50, 100, and 150 mg/mL.

Fourth, antibacterial activity testing using the Kirby-Bauer disc diffusion method. A suspension of *S. pneumoniae* was standardised to a turbidity equivalent to a 0.5 McFarland standard (≈1.5 x 10^8 CFU/mL). One millilitre of the suspension was evenly spread onto Nutrient Agar (NA) in a sterile petri dish. Sterile paper discs (6 mm diameter) impregnated with 10 µL of each extract concentration, pure DMSO (negative control), or a 5 µg ciprofloxacin solution (positive control) were placed onto the inoculated agar. Plates were incubated at 37°C for 18-24 hours. The diameter of the inhibition zone (clear area surrounding the disc) was measured using a digital calliper. Testing was performed in three independent replicates to ensure reliability.

Statistical data analysis was performed using IBM SPSS Statistics version 27. The normality of the data was assessed using the Shapiro-Wilk test. As the inhibition zone data were not normally distributed, comparisons among treatment groups were conducted using the non-parametric Kruskal-Wallis test. Where the Kruskal-Wallis result was significant, post-hoc pairwise comparisons were performed using the Mann-Whitney U test. The statistical significance level (α) was set at 0.05.

Results

Qualitative phytochemical screening of the Ashitaba leaf ethanol extract indicated positive results for all six classes of compounds tested. The detection results for each compound class are presented in Table 1. The presence of flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids indicates that the extract possesses a complex phytochemical profile rich in potentially bioactive secondary metabolites.

The disc diffusion assay demonstrated that the Ashitaba leaf ethanol extract inhibited the growth of *Streptococcus pneumoniae* at all tested concentrations. A clear zone formed around discs impregnated with the extract. The diameter of the inhibition zones exhibited an increasing trend with higher extract concentrations. The positive control (ciprofloxacin) produced the largest and most consistent inhibition zones, while the negative control (pure DMSO) showed no inhibition zone, confirming the solvent's lack of

antibacterial activity. The mean inhibition zone diameter from three replicates for each treatment is presented in Table 2.

Table 1. Results of qualitative phytochemical screening of ashitaba leaf ethanol extract

No.	Compound Class	Test Result	Positive Reaction Indicator
1	Flavonoids	Positive (+)	Formation of a red/orange colour in the amyl alcohol layer upon addition of Mg and concentrated HCl.
2	Alkaloids	Positive (+)	Formation of an orange to brown precipitate upon addition of Dragendorff's reagent.
3	Saponins	Positive (+)	Formation of stable foam persisting for more than 10 minutes after vigorous shaking.
4	Tannins	Positive (+)	Colour change to dark green or bluish-black upon addition of 1% $FeCl_3$ solution.
5	Glycosides	Positive (+)	Formation of a purple ring at the interface of two liquid layers upon addition of Molisch's reagent and concentrated H_2SO_4.
6	Steroids/Triterpenoids	Positive (+)	Formation of a green colour upon addition of Liebermann-Burchard reagent, indicating the presence of steroids.

Table 2. Mean inhibition zone diameter of ashitaba leaf extract and controls against *Streptococcus pneumoniae*

Treatment	Mean Inhibition Zone Diameter (mm)	Standard Deviation (mm)	Activity Category*
Extract 50 mg/mL	16.57	0.95	Moderate
Extract 100 mg/mL	26.23	1.35	Strong
Extract 150 mg/mL	29.87	1.4	Very Strong
Positive Control (Ciprofloxacin)	53.67	0.58	-
Negative Control (DMSO)	0.00	0.00	None

*Category adapted for herbal extracts based on inhibition zone diameter: <10 mm (weak), 10-20 mm (moderate), >20-30 mm (strong), >30 mm (very strong).

The Shapiro-Wilk test for normality on inhibition zone data from all treatment groups yielded a significance value of p=0.021 ($p < 0.05$), indicating non-normal distribution. Consequently, inferential statistical analysis proceeded with non-parametric tests. The Kruskal-Wallis test comparing all four groups (three extract concentrations and one positive control) revealed a statistically significant difference in median values (Chi-Square=10.421, p=0.015). To identify specific differences between pairs of groups, a post-hoc Mann-Whitney U test was performed.

Table 3. Results of non-parametric statistical tests for inhibition zone diameter comparisons

Group Comparison	p-value (Mann-Whitney U Test)	Statistical Decision (α=0.05)
Extract 50 mg/mL vs. 100 mg/mL	0.05	Not significantly different
Extract 50 mg/mL vs. 150 mg/mL	0.05	Not significantly different
Extract 100 mg/mL vs. 150 mg/mL	0.05	Not significantly different
Extract 50 mg/mL vs. Positive Control (Ciprofloxacin)	0.046	Significantly different
Extract 100 mg/mL vs. Positive Control (Ciprofloxacin)	0.046	Significantly different
Extract 150 mg/mL vs. Positive Control (Ciprofloxacin)	0.046	Significantly different

The results indicated no statistically significant difference in inhibition zone diameters among the three Ashitaba extract concentrations. Comparisons between 50 mg/mL and 100 mg/mL, 50 mg/mL and 150 mg/mL, and 100 mg/mL and 150 mg/mL all yielded a p-value of 0.050, precisely at the α=0.05 significance threshold. This suggests that, within the context of this study, increasing the extract concentration from 50 mg/mL to 150 mg/mL did not produce a statistically significant increase in inhibitory effect. In contrast, when each extract concentration was compared separately with the positive control ciprofloxacin, all comparisons showed a highly significant difference (p=0.046 for 50 mg/mL vs. ciprofloxacin, 100 mg/mL vs. ciprofloxacin, and 150 mg/mL vs. ciprofloxacin; $p < 0.05$). This conclusively demonstrates that while the Ashitaba leaf extract possesses antibacterial activity, its inhibitory potency against *S. pneumoniae* remains significantly lower than that of the synthetic antibiotic ciprofloxacin. A summary of the statistical test results is provided in Table 3.

Discussion

This study successfully demonstrates that the ethanol extract of Ashitaba leaves (*Angelica keiskei*) possesses a complex phytochemical composition and exhibits significant *in vitro* antibacterial activity against *Streptococcus pneumoniae*. The presence of various compound classes, including flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids, aligns with previous reports stating that Ashitaba is rich in bioactive compounds, particularly chalcone-type flavonoids such as xanthoangelol and 4-hydroxyderricin.[14] These compounds are known to exert antimicrobial effects through various mechanisms, including disruption of cell membrane integrity, inhibition of protein synthesis, and interference with essential bacterial enzymes.[15] The combination of these bioactive constituents is suspected to act synergistically, producing the observed antibacterial effect, where a multi-target mechanism may hinder the development of bacterial resistance.

The finding that Ashitaba extract actively inhibits *S. pneumoniae* reinforces and extends the results of prior studies that typically tested skin bacteria such as *Staphylococcus*. This provides preliminary scientific justification for the empirical use of Ashitaba and reveals its potential application against respiratory tract infections. The descriptive trend of increasing inhibition zone diameter with concentration is a common pharmacological phenomenon, illustrating a dose-response relationship where higher concentrations of active compounds elicit a greater biological effect.[16] However, the statistical analysis indicating no significant difference among the three concentrations (50, 100, and 150 mg/mL) warrants consideration. This phenomenon may be explained by several possibilities. First, a plateau effect may have occurred, whereby at 50 mg/mL, the active components of the extract had already reached or approached saturation in terms of their diffusion capacity within the agar medium, so further concentration increases did not linearly expand the diffusion zone.[17] Second, inherent limitations of the agar diffusion method itself in detecting subtle differences, particularly for compounds with high molecular weight or specific solubility. Third, the relatively small sample size (n=3 per group), despite low variability between replicates, may have reduced the statistical power to detect existing differences.

Although promising, the efficacy of the Ashitaba extract remained substantially inferior to the standard antibiotic ciprofloxacin. This is a common reality in herbal drug development research. Ciprofloxacin is a pure synthetic compound specifically designed for high affinity to its targets (bacterial DNA gyrase and topoisomerase IV), optimal bioavailability, and stability.[18] In contrast, a herbal extract is a complex mixture with lower concentrations of individual active compounds and may contain components that impede the penetration or activity of the antimicrobial constituents. This disparity does not necessarily diminish the potential value of Ashitaba extract but places it within a different application context. Ashitaba extract could be considered as an adjuvant therapy, a prophylactic agent, or a treatment for mild to moderate infections, or as a raw material for the isolation and modification of pure active compounds whose potential may equal or even exceed that of conventional antibiotics following optimisation processes.

Compared to other medicinal plants previously studied for activity against *S. pneumoniae*, Ashitaba extract demonstrates competitive performance. A study by Mindress (2021) testing local medicinal plants reported varying inhibition zones, with Ashitaba extract at 150 mg/mL producing a zone of nearly 30 mm, placing it in the strong category.[19] However, direct comparisons must be made cautiously due to differences in extraction methodology, solvents, and material standardisation. A strength of the present study is its focus on a clinically significant respiratory pathogen. For future research, it is important to proceed beyond diffusion tests to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) to quantitatively understand the extract's potential, to test against clinical strains of *S. pneumoniae* including antibiotic-resistant (MDR) strains, and to evaluate its toxicity against mammalian cells to ensure safety. Exploring more advanced formulations, such as nanoparticles incorporating Ashitaba extract as reported in some studies, could also be a strategy to enhance the stability, penetration, and bioavailability of its active compounds.[20]

Conclusion

Based on the study results, it is concluded that the ethanol extract of Ashitaba leaves (*Angelica keiskei*) contains phytochemical compounds from the classes of flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids. The extract exhibits *in vitro* antibacterial activity against *Streptococcus pneumoniae*, with inhibition zone diameters increasing with concentration, although no significant difference was observed among the 50, 100, and 150 mg/mL concentrations. The antibacterial activity of the Ashitaba

extract remains significantly lower than that of the antibiotic ciprofloxacin. Ashitaba leaf extract holds potential for further development as a source of natural antimicrobial agents. It is recommended that subsequent research determine the MIC/MBC, test against clinical strains, and evaluate the extract's toxicity and formulation.

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