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Original Article

Chronic tonsillitis and its potential alternative treatment: Exploration of the antibacterial activity of ethanolic *Mentha piperita* leaf extract against bacterial isolates from patients

Puti G. Munirwan[1*], Lily Setiani[1,2], Benny Kurnia[1,2], Novina Rahmawati[1,2] and Azwar Ridwan[1,2]

[1]Department of Otorhinolaryngology–Head and Neck Surgery, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [2]Department of Otorhinolaryngology–Head and Neck Surgery, Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia

*Corresponding author: putighina@gmail.com

Abstract

Chronic tonsillitis is commonly associated with persistent bacterial infection, predominantly involving Gram-positive organisms, and increasing antimicrobial resistance has encouraged exploration of alternative therapeutic agents derived from medicinal plants. Mint leaves (*Mentha piperita*) are known to contain bioactive compounds with potential antibacterial activity; however, their antibacterial efficacy against isolates specifically obtained from patients with chronic tonsillitis has not been clearly established. The aim of this study was to evaluate the antibacterial activity of ethanolic *M. piperita* leaf extract against bacterial isolates from patients with chronic tonsillitis. An experimental laboratory study was conducted using bacterial isolates from tonsillar core specimens. Antibacterial activity was assessed using the disk diffusion method at various extract concentrations, followed by determination of the minimum inhibitory concentration (MIC) by the microdilution method and the minimum bactericidal concentration (MBC) by subculture on plate count agar. Results showed that Gram-positive bacteria were the predominant isolates, with *Staphylococcus aureus* identified as the most frequent species, followed by *Streptococcus agalactiae* and *Pseudomonas aeruginosa*. The extract demonstrated greater inhibitory activity against Gram-positive bacteria, with the largest inhibition zone observed in *Streptococcus agalactiae* at 50% concentration (18.31 mm). In the microdilution assay, the lowest tested concentration (0.75%) inhibited bacterial growth in all tested species, and no colony growth was observed upon subculture, indicating bactericidal activity at this concentration. These findings suggest that ethanolic *M. piperita* leaf extract exhibits antibacterial activity, particularly against Gram-positive pathogens associated with chronic tonsillitis, and may represent a potential natural antimicrobial agent for further investigation.

Keywords: Chronic tonsillitis, *Mentha piperita*, antibacterial activity, traditional medicine, bacterial isolates

Introduction

Infectious diseases remain a major global health concern, with antibiotic resistance posing a serious threat to effective treatment, including in otorhinolaryngology conditions such as

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tonsillitis [1]. Tonsillitis is an inflammatory disease of the palatine tonsils, part of Waldeyer's ring, and chronic tonsillitis is associated with recurrent infection, host and environmental factors, and inadequate antibiotic use, presenting with chronic sore throat, halitosis, lymphadenopathy, and tonsillar structural changes [2]. The prevalence of tonsillitis varies globally, with millions of cases diagnosed annually in the United States and France, and it represents a substantial proportion of primary care consultations [3,4]. In Indonesia, a 2012 epidemiological survey reported a tonsillitis prevalence of 3.8%, ranking second among otorhinolaryngology diseases [5], and its incidence has increased over time in some hospitals [6].

The bacterial etiology of chronic tonsillitis varies by region. *Streptococcus pyogenes* accounts for 15%–36% of cases globally, while studies from Tanzania, Saudi Arabia, and Indonesia reported *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Pseudomonas aeruginosa* as predominant pathogens isolated from tonsillar samples [7-9]. Several other studies also demonstrated low sensitivity to commonly used antibiotics, and the increasing emergence of multidrug-resistant organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESβL)-producing Gram-negative bacteria, contributes to treatment failure, recurrent infection, and the need for tonsillectomy [1,7,10-12].

Given the increasing antibiotic resistance and limited effectiveness of conventional therapy, alternative plant-derived antimicrobial agents are being explored [13,14]. Mint leaves (*Mentha piperita*) contain bioactive compounds such as flavonoids, polyphenols, tannins, and menthol, which exhibit antibacterial effects by disrupting bacterial cell membranes and inhibiting protein synthesis [15,16]. However, evidence regarding the antibacterial activity of *M. piperita* against bacterial isolates specifically obtained from chronic tonsillitis remains limited. The aim of this study was to evaluate the antibacterial activity of the ethanol extract of *M. piperita* leaves against *S. aureus*, *S. agalactiae*, and *P. aeruginosa* isolated from chronic tonsillitis.

Methods

Study design and setting

An experimental laboratory study with a posttest-only control group design was conducted at Dr. Zainoel Abidin Hospital and Universitas Syiah Kuala, in Banda Aceh, Indonesia, from July to October 2025. The study included multiple treatment groups corresponding to different concentrations of ethanolic *M. piperita* extract, which were tested against bacterial isolates to assess antibacterial activity through disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays.

Bacterial isolates and identification

Bacterial isolates were obtained from patients with chronic tonsillitis undergoing tonsillectomy at Dr. Zainoel Abidin Hospital. Following surgical excision, tonsillar tissues were briefly immersed in povidone–iodine solution for surface decontamination and rinsed with sterile 0.9% sodium chloride solution. The tonsillar tissue was then aseptically sectioned, and approximately 0.5 mm of the tonsillar core was collected and inoculated into brain heart infusion broth (BHIB) before being transported immediately to the hospital's Clinical Microbiology Laboratory for culture.

After incubation at 35±2°C for 24 hours, samples showing bacterial growth were subcultured onto blood agar and MacConkey agar plates using the quadrant streak method and incubated under the same conditions. Bacterial identification was performed based on macroscopic colony characteristics, Gram staining, and further species-level identification using the VITEK 2 Compact automated system (bioMérieux, Marcy-l'Étoile, France).

A total of 11 tonsillar core specimens were collected, of which 7 yielded pathogenic bacterial growth, resulting in 8 bacterial isolates. Of these isolates, *S. aureus, S. agalactiae*, and *P. aeruginosa* were selected for antibacterial testing. Each of these species was represented by one clinical isolate recovered from the tonsillar core culture. All antibacterial assays were performed in technical triplicate for each isolate.

Plant material and extraction

Fresh *M. piperita* leaves were collected from the Neusu area, Baiturrahman District, Banda Aceh, Indonesia. Mature, dark-green leaves that were intact and free from visible damage were selected, while apical leaves were excluded to avoid overly young plant material. Botanical identification of the plant material was confirmed through herbarium examination at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala (voucher: 863iUN11.F8.4/TA.00.03/2025).

The leaves were separated from the stems, washed thoroughly with running water, and dried in a drying cabinet at 45°C until a constant weight was achieved. The dried leaves were then subjected to dry sorting, pulverized into fine powder, and stored in airtight containers protected from direct light. Extraction was performed using the maceration method with 70% ethanol as the solvent. The macerate was filtered, and the filtrate was concentrated by rotary evaporation at 60°C to obtain a thick ethanolic extract of *M. piperita*.

Preparation of extract concentrations and bacterial inoculum

A stock solution of ethanolic *M. piperita* extract was prepared by dissolving 10 g of the concentrated extract in distilled water to a final volume of 10 mL, yielding a 100% (w/v) concentration. Serial dilutions were subsequently prepared from the stock solution to obtain extract concentrations of 50%, 25%, 12.5%, 6.25%, 6%, 3%, 1.5%, and 0.75%, with each concentration adjusted to a final volume of 10 mL. Dilutions were calculated using the standard dilution formula ($C_1V_1=C_2V_2$). All prepared extract solutions were stored in light-protected containers wrapped in aluminum foil at temperatures below 4°C until use.

Bacterial isolates obtained from chronic tonsillitis specimens were retrieved from stock cultures and subcultured onto blood agar plates. After incubation for 24 hours, well-isolated colonies were suspended in sterile 0.9% sodium chloride solution. The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard to ensure a standardized inoculum for antibacterial testing.

Antibacterial activity assay

Antibacterial activity of the ethanolic *extract of M. piperita* was evaluated at the Microbiology and Infection Laboratory, Faculty of Medicine, Universitas Syiah Kuala. Standardized bacterial suspensions (0.5 McFarland) were used, and all assays were performed in triplicate. For the disk diffusion assay, Mueller–Hinton agar plates were inoculated using a sterile cotton swab, and sterile blank Kirby–Bauer disks were impregnated with each extract concentration as well as positive (amoxicillin 25 µg) and negative (96% ethanol) controls. Plates were incubated at 37 °C for 24 hours, after which inhibition zones were measured with a vernier caliper and categorized according to Davis and Stout (<5 mm weak, 5–10 mm moderate, 10–20 mm strong, >20 mm very strong).

The MIC was determined using a microdilution method in 96-well flat-bottom microplates containing Mueller–Hinton broth, the extract at 6%, 3%, 1.5%, and 0.75%, and 10 µL of a standardized bacterial suspension. Media control wells (Mueller–Hinton broth only), growth control wells (broth with bacterial suspension without extract), and antibiotic control wells (amoxicillin) were included in each assay. Plates were covered with parafilm, incubated at 37°C for 18–25 hours, and bacterial growth was assessed by measuring optical density (OD) with an enzyme-linked immunosorbent assay (ELISA) reader. OD values were recorded as mean ± standard deviation from triplicate wells and were used as an indirect indicator of bacterial growth. Because the extract possessed intrinsic color and turbidity, the OD-based findings were interpreted cautiously and were further considered together with the subculture results. The lowest extract concentration inhibiting visible bacterial growth was recorded as the MIC.

For MBC determination, aliquots (10 µL) from wells showing no visible growth in the MIC assay were subcultured onto plate count agar plates and incubated at 37°C for 24 hours. After incubation, bacterial colonies were counted and expressed as colony-forming units (CFU). The MBC was defined as the lowest extract concentration resulting in no recoverable bacterial colonies (zero CFU) on plate count agar.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, NY, USA). Inhibition zone diameters obtained from the disk diffusion assay were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA). When significant differences were observed, Duncan's post-hoc test was applied to identify differences among extract concentrations. A significance level of $p<0.05$ was used. MIC and MBC results were determined descriptively based on optical density measurements and colony growth on plate count agar, respectively, without inferential statistical analysis.

Results

Culture and bacterial isolate distribution

A total of eleven tonsillar core tissue specimens were obtained from patients diagnosed with chronic tonsillitis who underwent tonsillectomy. Microbiological culture examination demonstrated that seven specimens (63.6%) showed growth of pathogenic bacteria, while four specimens (36.4%) showed no pathogenic bacterial growth (**Table 1**).

Table 1. Tonsillar core culture results from patients with chronic tonsillitis who underwent tonsillectomy

Culture result	Frequency	Percentage (%)
Positive for pathogenic bacteria	7	63.6
Negative for pathogenic bacteria	4	36.4
Total	11	100

Among the specimens with positive bacterial growth, both Gram-positive and Gram-negative bacteria were identified. Gram-positive bacteria predominated, accounting for 75% of the total isolates, whereas Gram-negative bacteria constituted 25%. One specimen exhibited polymicrobial growth, while the remaining specimens showed single bacterial growth **(Table 2)**. Consequently, a total of eight bacterial isolates were recovered from seven culture-positive specimens. Based on species-level identification, *S. aureus* was the most frequently isolated organism. Other Gram-positive bacteria identified included *S. agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus mitis*. Gram-negative isolates consisted of *P. aeruginosa* and *Sphingomonas paucimobilis* (**Table 2**). Only *S. aureus*, *S. agalactiae*, and *P. aeruginosa* were included for further analysis because they were considered the most clinically relevant to the study objective, representing the predominant isolate in the present study (*S. aureus*), other pathogenic Gram-positive bacteria (*S. agalactiae*), and a representative Gram-negative pathogen (*P. aeruginosa*) associated with chronic tonsillitis.

Table 2. Distribution of bacterial isolates recovered from tonsillar core cultures of patients with chronic tonsillitis who underwent tonsillectomy

Bacterial group	Species	Frequency	Percentage (%)
Gram-positive bacteria	*Staphylococcus aureus*	3	37.5
	Streptococcus agalactiae	1	12.5
	Streptococcus dysgalactiae	1	12.5
	Streptococcus mitis	1	12.5
	Subtotal	6	75.0
Gram-negative bacteria	*Sphingomonas paucimobilis*	1	12.5
	Pseudomonas aeruginosa	1	12.5
	Subtotal	2	25.0
Total isolates		8	100

Disk diffusion assay

In *S. aureus*, the maximum inhibitory effect was observed at an extract concentration of 25%, producing an inhibition zone of 13.57 mm, and a slight reduction in inhibition was noted at 50% (12.43 mm) (**Table 3**). For *S. agalactiae*, inhibition zone diameters increased consistently with rising extract concentrations, from 11.24 mm at 6.25% to 18.31 mm at 50% (**Table 3**). The largest inhibition zone was observed at 50%, indicating strong antibacterial activity, although it

remained lower than that of the positive control amoxicillin (26.98 mm). In contrast, *P. aeruginosa* exhibited minimal inhibition across all extract concentrations, with inhibition zone diameters ranging from 6.11 to 7.14 mm (**Table 3**). These values were comparable to those of the negative control (6.00 mm) and the positive control (6.63 mm), indicating limited antibacterial activity of the extract against this Gram-negative bacterium (**Table 3**).

Table 3. Mean inhibition zone diameters of ethanolic *Mentha piperita* leaf extract against bacterial isolates from patients with chronic tonsillitis who underwent tonsillectomy

Bacterial species	*Mentha piperita* extract concentration (%), mm				Positive control, mm	Negative control, mm
	6.25	12.5	25	50		
Staphylococcus aureus	6.10	10.39	13.57	12.43	6.93	6.00
Streptococcus agalactiae	11.24	13.07	17.62	18.31	26.98	6.00
Pseudomonas aeruginosa	6.55	7.14	6.47	6.11	6.63	6.00

Minimum inhibitory concentration (MIC) results

For *S. aureus*, extract concentrations of 0.75% and 1.5% showed OD values lower than the growth control, indicating inhibition of bacterial growth at these concentrations (**Table 4**). Similar findings were observed for *S. agalactiae*, where reduced OD values compared with the growth control were noted at 0.75% and 1.5% (**Table 4**). In *P. aeruginosa*, OD values at extract concentrations ranging from 0.75% to 3% were lower than those of the growth control, suggesting growth inhibition at these concentrations (**Table 4**). Based on the absence of observable growth relative to the growth control, the MIC of the ethanolic *M. piperita* extract for all tested bacterial species was determined to be 0.75%, the lowest concentration evaluated in this study (**Table 4**). However, because plant extracts may contribute background color and turbidity that influence spectrophotometric readings, this value should be interpreted as an apparent inhibitory concentration under the conditions of the present study.

Table 4. Optical density values from the minimum inhibitory concentration (MIC) microdilution assay of ethanolic *Mentha piperita* extract against bacterial isolates

Bacterial species	Optical density, mean±SD						
	Mentha piperita extract				Growth control	Media control	Antibiotic control
	0.75%	1.5%	3%	6%			
Staphylococcus aureus	0.106 ± 0.001	0.171 ± 0.007	0.318 ± 0.034	0.591 ± 0.017	0.331 ± 0.042	0.043 ± 0.001	0.043 ± 0.003
Streptococcus agalactiae	0.113 ± 0.003	0.171 ± 0.006	0.292 ± 0.020	0.305 ± 0.049	0.157 ± 0.032	0.043 ± 0.001	0.046 ± 0.000
Pseudomonas aeruginosa	0.103 ± 0.004	0.172 ± 0.006	0.311 ± 0.020	0.620 ± 0.032	0.738 ± 0.025	0.044 ± 0.001	0.046 ± 0.001

Minimum bactericidal concentration (MBC) results

Across all tested bacterial species, no recoverable colonies were observed on plate count agar at extract concentrations ranging from 0.75% to 6% (**Table 5**). In contrast, substantial bacterial growth was consistently detected in the growth control, confirming the viability of the bacterial inocula under the experimental conditions. Specifically, the growth control yielded 238–247 CFU for *S. aureus*, 40–49 CFU for *S. agalactiae,* and 130–150 CFU for *P. aeruginosa*. No colony growth was observed in the media control or antibiotic control wells (**Table 5**). These results suggest that the ethanolic *M. piperita* extract exerted bactericidal activity against all tested bacterial species at concentrations as low as 0.75%.

Table 5. Minimum bactericidal concentration (MBC) of ethanolic *Mentha piperita* extract against bacterial isolates

Bacterial species	Colony-forming units (CFU)						
	Mentha piperita extract				Media control	Antibiotic control	Growth control
	0.75%	1.5%	3%	6%			
Staphylococcus aureus	0	0	0	0	0	0	238–247
Streptococcus agalactiae	0	0	0	0	0	0	40–49
Pseudomonas aeruginosa	0	0	0	0	0	0	130–150

Discussion

This study evaluated the antibacterial activity of ethanolic *M. piperita* leaf extract against bacterial isolates obtained from patients with chronic tonsillitis. Pathogenic bacteria were recovered from 63.6% of tonsillar core specimens, with Gram-positive organisms predominating. *S. aureus* was the most frequently isolated species, followed by *S. agalactiae* and *P. aeruginosa*. The extract demonstrated measurable antibacterial activity in disk diffusion assays, particularly against Gram-positive isolates. In the microdilution assay, the lowest tested concentration (0.75%) inhibited bacterial growth in all tested species, and no recoverable colonies were observed on subculture, indicating bactericidal activity at this concentration.

The predominance of Gram-positive bacteria in this study is consistent with previous reports describing *S. aureus* and streptococcal species as common pathogens in chronic tonsillitis [6,17]. Similar culture positivity rates have also been documented in earlier studies, although variability may occur depending on sampling technique, prior antibiotic exposure, and the absence of anaerobic culture facilities [18,19]. The isolation pattern observed in this study supports the relevance of targeting Gram-positive organisms when exploring alternative antimicrobial agents for chronic tonsillitis.

In the disk diffusion assay, the extract exhibited greater inhibitory effects against Gram-positive bacteria than against the Gram-negative isolate. *S. agalactiae* demonstrated a concentration-dependent increase in inhibition zone diameter, reaching 18.31 mm at 50%, whereas *S. aureus* showed optimal inhibition at 25%. In contrast, *P. aeruginosa* exhibited minimal inhibition across all concentrations, with zone diameters comparable to controls. This differential susceptibility aligns with established structural differences between Gram-positive and Gram-negative bacteria [20]. The outer membrane of Gram-negative bacteria, enriched with lipopolysaccharides, acts as an additional permeability barrier that can restrict the penetration of plant-derived bioactive compounds [19,21-23].

The antibacterial activity observed in this study may be attributed to the presence of bioactive phytochemicals in *M. piperita*, including tannins, alkaloids, phenolic compounds, terpenoids, and saponins [24,25]. These secondary metabolites are known to exert antibacterial effects through multiple mechanisms, including disruption of bacterial cell membranes, alteration of membrane permeability, inhibition of enzymes, and interference with protein synthesis [26-28]. Phenolic and terpenoid components, including menthol derivatives, are particularly recognized for their ability to destabilize bacterial lipid bilayers, leading to leakage of intracellular contents and cell death [29].

The microdilution assay demonstrated that extract concentrations of 0.75% inhibited bacterial growth relative to the growth control in all tested species. Although optical density values increased at higher concentrations in some groups, this pattern may be influenced by the intrinsic color and light-absorbing properties of concentrated plant extracts, which can interfere with spectrophotometric measurements [30]. Such interference has been reported in previous studies evaluating ethanolic extracts of *M. piperita*, underscoring the importance of confirming inhibitory findings with culture-based assays [31,32].

Consistent with the MIC findings, subculture onto plate count agar revealed no recoverable colonies at concentrations ranging from 0.75% to 6% for all tested bacteria. The absence of colony growth, contrasted with substantial growth in the untreated control wells, indicates bactericidal activity at the lowest concentration evaluated. Previous studies have reported comparable MIC ranges for *M. piperita* extracts against oral and respiratory pathogens, particularly demonstrating stronger effects against Gram-positive bacteria than Gram-negative species [33-35]. The findings of the present study, therefore, support the potential antibacterial role of M. piperita against pathogens implicated in chronic tonsillitis.

Beyond its direct antibacterial action, *M. piperita* has been reported to possess anti-inflammatory and antibiofilm properties that may be relevant in chronic tonsillar infection [36-38]. Chronic tonsillitis is often associated with persistent inflammation and biofilm formation, which contribute to recurrent infection and reduced antibiotic responsiveness [22]. The combined antimicrobial and anti-inflammatory properties of peppermint-derived compounds may therefore offer therapeutic advantages, particularly as adjunctive or supportive interventions [39].

This study has several limitations. The sample size was small, with only 11 tonsillar core specimens analyzed, and only 7 specimens yielded pathogenic bacterial growth. Although eight bacterial isolates were recovered, antibacterial testing was performed only on *S. aureus, S. agalactiae*, and *P. aeruginosa*, each represented by a single clinical isolate, with assays performed in technical triplicate. Therefore, the findings should be regarded as preliminary and may not reflect the full variability in susceptibility among strains or across all bacterial species identified. In addition, only aerobic culture was performed, which may have underestimated the contribution of anaerobic pathogens. The MIC findings were based on optical density measurements that may have been influenced by the intrinsic color and turbidity of the extract, whereas the MBC findings should be interpreted cautiously because no neutralization step was performed before subculture. Furthermore, phytochemical characterization was not quantitatively standardized, and several methodological standardization steps, including extract sterility verification and confirmatory susceptibility procedures, were not fully established. Accordingly, further studies using larger numbers of isolates, standardized extract characterization, and more rigorous antimicrobial testing procedures are needed.

Conclusion

This study demonstrated that ethanolic *M. piperita* leaf extract exhibits antibacterial activity against bacterial isolates obtained from patients with chronic tonsillitis. Gram-positive bacteria were the predominant isolates, with *S. aureus* identified as the most frequent species. The extract showed measurable inhibitory effects in the disk diffusion assay, particularly against Gram-positive organisms, and exhibited inhibitory and bactericidal activity at the lowest tested concentration (0.75%) in the microdilution and subculture assays. These findings suggest that *M. piperita* extract may serve as a natural antimicrobial agent against pathogens associated with chronic tonsillitis and warrant further exploration of its potential therapeutic applications.

Ethics approval

The study protocol was reviewed and approved by the Ethics Committee of the Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia (Approval No.183/ETIK-RSUDZA/2025).

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study utilized artificial intelligence (AI) language model, ChatGPT, to improve grammar, sentence structure, and readability. The authors critically reviewed and revised all AI-generated outputs to ensure accuracy, coherence, and alignment with the study's objectives. The final decisions, interpretations, and manuscript content reflect the authors' independent judgment and intellectual contributions.

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