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Antibacterial Effect of Honey Against *Pseudomonas aeruginosa* in Chronic Suppurative Otitis Media Patients and ATCC Bacterial Strains in Vitro

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Abstract: *Pseudomonas aeruginosa* and *Staphylococcus aureus* are common causative bacteria in *Chronic Suppurative Otitis Media* (CSOM). Honey, as a natural substance, has shown antibacterial activity. To evaluate the antibacterial effect of honey at different concentrations on *P. aeruginosa* (clinical isolates and ATCC 27852) and *S. aureus* (ATCC 29213) compared to ofloxacin. An experimental in vitro study using post-test only design was conducted. Bacterial isolates from CSOM patients and ATCC strains were exposed to honey at concentrations of 60%, 70%, 80%, 90%, and 100%. Zone of inhibition was measured using the agar diffusion method. Honey exhibited significant antibacterial activity against all tested bacteria. The highest inhibition zones were observed at 100% concentration. Ofloxacin showed greater inhibition than honey in general, but honey still demonstrated moderate to strong activity. Honey has potential as a natural antibacterial agent against *P. aeruginosa* and *S. aureus*, and may serve as an alternative or adjunct treatment for CSOM.

Keywords: Antibacterial, CSOM, honey, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, zone of inhibition.

Introduction

Chronic suppurative otitis media (CSOM) is one of the most common infectious diseases encountered in otolaryngology practice, affecting children worldwide, regardless of geographic or socioeconomic status (Khairkar, 2023). Otitis media itself has many types, namely acute otitis media (AOM), recurrent AOM, AOM with effusion, chronic suppurative otitis media (CSOM), and middle ear effusion (ETE) (Schilder et al., 2016). Otitis media is the most common ear disease experienced by children (<15 years) (Fasunla et al., 2013). One of the most common types of otitis media is CSOM.

CSOM is a chronic inflammation of the middle ear and mastoid for more than 6 weeks with typical signs of tympanic membrane perforation and otorrhea (Gustada, Hafil., 2020). Other literature states that chronic conditions occur when complaints have been present for more than 2 weeks (Schilder et al., 2016). The incidence of CSOM is estimated at more than 20 million people worldwide. In industrialized countries, it is revealed that approximately 80% of preschool children may have experienced at least one episode of acute otitis media (AOM) before they are three years old, and nearly 40% will experience six or more recurrences by the time they reach the age of seven (Khairkar, 2023). In a study conducted by Hunt et al. (2017) on the examination of 281 children (aged 4-6 years), it was found that 5.3% of children experienced CSOM, followed by serous otitis media at 3.6% and AOM at 2.8%. In other studies, the percentage of OMSK cases in adults reached 10.9% of all ear disorders (Fasunla et al., 2013).

CSOM is generally caused by microbial infections. The most common bacterial infections causing CSOM are *Pseudomonas aeruginosa* (21.01%-38% of cases) and *Staphylococcus aureus* (5.88%-29% of cases) (Asroel et al., 2013; Wahida et al., 2016). One of the obstacles in managing CSOM is the presence of antibiotic-resistant bacterial infections. From various studies in Indonesia, antibiotic sensitivity testing on P. aeruginosa bacteria found resistance to amoxicillin-clavulanate (13-100% of isolates), clindamycin (100% of isolates), and ciprofloxacin (30.77-38%). Resistance to amoxicillin-clavulanate (25% of isolates), clindamycin (30%-75% of isolates), and ciprofloxacin (10% of isolates) has been found in *S. aureus* (Dewi, 2013; Rumimpunu et al., 2014; Wahida et al., 2016).

The presence of these resistant microbes can increase the risk of complications and poor treatment outcomes (Mushi et al., 2016). To address these resistant microbes, new antibacterial alternatives, including herbal therapies, are needed. Honey may be a solution or natural ingredient with potential as a new antibacterial to treat infections in CSOM.

The use of honey for medicinal purposes has been known for over 4,000 years. Hippocrates described honey for treating burns and abscesses, while Dioscorides (a Roman physician) used honey to heal wounds and inflammation of the throat and tonsils (Werner, Laccourreye, 2011). The use of honey has been studied in various ear conditions, such as recurrent otitis externa and otitis media (Tharakan et al., 2018).

Honey is an example of an acidic product (pH 4) with various bioactive substances depending on the flower nectar consumed by bees. On average, honey contains 80% various sugars, 17-20% water, and 4% other substances, as well as various polyphenols, vitamins, and volatile substances (Werner, Laccourreye, 2011). Honey's antibacterial properties are thought to stem from its acidic nature, low water content, hydrogen peroxide, and other non-peroxide antibacterial compounds. This combination of various antibacterial substances is what makes researchers believe that honey can treat various bacterial infections, both Gram-positive and Gram-negative (Albaridi., 2019).

Various studies have used honey to test its antibacterial effects against S. aureus and P. aeruginosa bacteria. Using White honey and Amber honey at 50% concentrations, respectively, produced inhibition zones of 36.5 mm and 31.5 mm against *S. aureus* bacteria. Against P. aeruginosa, White honey and Amber honey produced inhibition zones of 15.6 mm and 16.3 mm, respectively (Ashari et al., 2020). In other studies, honey produced inhibition zones ranging from 9±2 mm to 27±1 mm against *S. aureus*, and from no inhibition zone to 16±1 mm against P. aeruginosa (Anthimidou, Mossialos, 2013). Based on the descriptions above, researchers were interested in understanding honey's antibacterial properties and its antibacterial effectiveness compared to ofloxacin against the bacteria that cause chronic suppurative otitis media.

Method

Experimental design

This type of research is an in vitro laboratory experimental study using a post-test design to see the effect of honey to suppress the growth of *Pseudomonas aeruginosa* bacteria in patients with chronic suppurative otitis media (CSOM), *P. aeruginosa* ATCC 27852 and *Staphylococcus aureus* ATCC 29213. Sampling was carried out at Murni Teguh Hospital, Medan. Examination of bacterial species and testing of antibacterial effectiveness were carried out at the Microbiology Laboratory of Prof. Dr. Chairudin P. Lubis Hospital. The target population in this study were patients with CSOM. The accessible population were patients with CSOM who were confirmed positive for *Psedumonas aureginosa* bacteria who were treated at Murni Teguh Hospital, Medan from November to December 2024.

Material and equipment

The research tools are laboratory glassware, petri dishes, incubators, autoclaves, analytical balances, spirit lamps, micropipettes, ose wire, aluminum foil, cork borers, and biosafety cabinets. The research materials are Indonesian honey, Mueller Hinton Agar (MHA) media, Mc.farland standard suspension, sterile NaCl solution, blood agar media, transport media (Amies agar transport), antibiotics, and distilled water.

Specimen preparation

Patients who came for treatment to the ENT Clinic diagnosed with CSOM were asked for prior consent to participate in the study. If the patient agreed, specimen collection was performed intraoperatively. 3. Patients did not receive antibiotic therapy 3 hours before specimen collection, if antibiotics had already been given, then the sample collection was carried out at least 6 hours without antibiotics. Specimens were taken using sterile cotton swabs in the middle ear and labeled with the patient's name and then stored in a tube containing transport media to be taken to the Microbiology Laboratory in less than 2 hours to maintain the quality and accuracy of the examination results.

Sample culture

The culture process to antibacterial effectiveness testing, the samples were conducted in a biosafety cabinet. The samples were then swabbed onto the surface of previously prepared blood agar media. The media containing the swabs was then incubated for 24 hours at 37°C in an incubator. The following day, the bacterial colonies were observed. The media containing the bacterial colonies was then examined using a Vitek 2 Compact to determine the bacterial species.

Honey preparation

Make honey extract, five concentrations of honey were prepared: 60%, 70%, 80%, 90%, and 100%. Pure honey (100%) was diluted using sterile distilled water. The dilution method was as follows:

$$C1 \times V1 = C2 \times V2 \quad \textbf{(1)}$$

Description:
C1 = initial concentration V1 = initial volume
C2 = final concentration V2 = final volume

Antibacterial activity

The antibacterial effect of honey was tested using the disc diffusion method. Blood agar media containing bacterial colonies was swabbed with a sterile loop. The loop was then swabbed onto Mueller-Hinton media. Six wells were then drilled into the Mueller-Hinton media (using a cork-borer) for each of the five honey concentrations and one for placing an antibiotic as a positive control. Approximately 50µL of each honey concentration and antibiotic was added to each well. The media was then incubated for 24 hours at 37°C. The following day, the diameter of the inhibition zone formed was observed and recorded. This antibacterial effectiveness test was conducted twice.

Data analysis

Data on the etiology of bacterial species are presented descriptively in the form of frequencies and percentages. Analysis of the antibacterial effectiveness of honey was performed using ANOVA if the distribution was normal; otherwise, using the Kruskal-Wallis test.

Results and discussion

Antibacterial activity test of treatment groups against *Pseudomonas aeruginosa* bacteria in CSOM patients

The full findings of measuring the inhibition zone diameter for each therapy group on the growth of *Psedumonas aeruginosa* bacteria from CSOM patients are shown in Table 1, which also shows the mean, standard deviation, median, minimum, and maximum values.

Tabel 1. Antibacterial activity test of treatment groups against *Pseudomonas aeruginosa* bacteria in CSOM patients

Group	Mean ± SD, mm	Median (Min – Max), mm
Control (-)	0	0
Control (+) (Ofloxacin)	25,5 ± 0,41	25,5 (25 – 26)
Honey 60 %	0	0
Honey 70 %	0	0
Honey 80 %	0	0
Honey 90 %	0	0
Honey 100 %	0	0

Only the control (+) group receiving Ofloxacin exhibited an average inhibition zone diameter of 25.5 mm, with the smallest diameter measuring 25 mm and the greatest measuring 26 mm, according to test results on the diameter of the inhibition zone. The inhibitory zone diameter was completely absent in the other groups.

Based on the treatment group, a significant difference in inhibition zone diameter was found using the Kruskal-Wallis test ($p < 0.001$). A post-hoc test was used to continue the research and identify whether groups displayed significant variations in inhibitory zone diameter. The positive control group and all other treatment groups showed a significant difference in inhibitory zone diameter ($p < 0.05$) according to the Dunn test. In contrast to the honey-treated group, the inhibition zone diameter did not differ between the negative control group and any of the treatment groups.

Tabel 2. Differences in Inhibition Zone Diameter Based on Treatment for *Pseudomonas aeruginosa* Bacterial Growth in CSOM Patients

Group	p	Posthoc[b]					
		C (+)	60%	70%	80%	90%	100%
Control (-)	<0,001[a]	0,002	1,000	1,000	1,000	1,000	1,000
Control (+)			0,002	0,002	0,002	0,002	0,002
Honey 60 %				1,000	1,000	1,000	1,000
Honey 70 %					1,000	1,000	1,000
Honey 80 %						1,000	1,000
Honey 90 %							1,000
Honey 100 %							

[a]Kruskal Wallis, [b]Dunn

According to a number of earlier investigations, such as the one conducted by Syawaluddin (2019), black honey can stop *Pseudomonas aeruginosa* bacteria from growing at concentrations ranging from 10% to 100%. *Pseudomonas aeruginosa* bacterial growth is effectively inhibited by a concentration of 80% black honey. Black honey's capacity to suppress *Pseudomonas aeruginosa* bacterial growth and the dose at which this effect can be observed are the two main objectives of this investigation. According to research by Purnama et al. (2024), the group that received a 12.5% propolis intervention had a mean OD (Optical Density) value that was 1.24 (p = 0.007) higher than the group that did not get propolis intervention (0%).

The 6.25% propolis intervention group also had a significantly higher mean OD value than the group that did not receive propolis intervention (0%), with a difference of 0.78 (p = 0.008). This means that administering 6.25% and 12.5% propolis will increase the ability of P. aeruginosa bacteria to form biofilms. The results also showed no significant difference in OD (Optical Density) values between the 3.125%, 1.56%, and 0% intervention groups (p>0.05). This shows that propolis administration at concentrations of 3.125% and 1.56% was unable to inhibit P. aeruginosa biofilm formation. Therefore, propolis can inhibit biofilm formation by 19.67% with a higher concentration of 0.78%. It can be concluded that propolis has antibiofilm activity against P. aeruginosa bacteria at a concentration of 0.78%. This study aims to test the antibiofilm activity of propolis in inhibiting *P. aeruginosa* biofilm formation.

Nurinsani (2019) study found that propolis and cefadroxil administration improved hearing by reducing air conduction by 7.09% compared to the control group and bone conduction by 13.06% compared to the control group. Sinulingga (2015) study found a p-value of 0.001 (p<0.05), indicating a significant relationship between the treatment and the observed results. The higher the dose of honey administered, the less likely it was that bacteria would grow. The contingency coefficient table showed a contingency coefficient of 0.678, indicating a strong relationship. Honey is concluded to have antibacterial effectiveness against Pseudomonas aeruginosa ATCC 27853 in vitro.

According to Tikonuwu *et al.,* (2024), the inhibition zone that developed against the test bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* had an average diameter of 6.83 mm and 8.58 mm, respectively. These findings placed the inhibition zone against these two bacteria in the category of moderate inhibition. Finding out how effective Halimeda opuntia algae extract is against *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria is the goal of this study. The algae extract was collected from the coastal waters of Poopoh Village, Tombariri District, Minahasa Regency. Halimeda is one of the algae genera in the Chlorophyta division that has the potential as a natural antibacterial and antioxidant.

The study by Putri and Maulida (2019) aims to assess the antibacterial activity of natural and processed honey at 25%, 50%, 75%, and 100% concentrations as well as to ascertain the antibacterial activity of each honey against the growth of *Staphylococcus aureus*. Antibacterial activity against *Staphylococcus aureus* was demonstrated by the administration of both natural and processed honey; the diameter of the inhibition zone for natural honey was 27, 29, 4, 32.1, and 36.2 mm, while that of processed honey was 6, 18.36, 30.33, and 33.66 mm. The positive control was 42.8 mm, and the negative control was 0 mm. The negative control was distilled water, and the positive control was amoxicillin antibiotic discs.

The findings of the Kruskal Wallis non-parametric test showed that both natural and processed honey had an inhibitory effect on the growth of *Staphylococcus aureus* germs ($p = 0.009$). The findings of the Mann-Whitney post hoc analysis showed that both natural and processed honey had inhibitory activity against *Staphylococcus aureus* at a range of concentrations (25, 50, 75, and 100)% ($p<0.05$). The test results also showed that the inhibitory activity of packaged honey differed significantly ($p<0.05$) from that of wild honey at 25% and 50% concentrations. Both natural and processed honey have the ability to suppress the growth of Staphylococcus aureus, however the inhibitory impact of natural honey is greater than that of processed honey.

Antibacterial activity test of treatment groups against *Pseudomonas aeruginosa* bacteria ATC 27852

Table 3 presents the complete results of the inhibition zone diameter measurements of each treatment group on the growth of Psedumonas aeruginosa ATCC 27852 bacteria by displaying the mean, standard deviation, median, minimum and maximum values. Using *Pseudomonas aeruginosa* ATCC 27852, it was reported that the control (+) group given Ofloxacin showed the largest inhibition zone diameter with an average of 30.88 mm, with the smallest diameter being 30 mm and the largest being 31.5 mm. Meanwhile, the 100% honey group had an average inhibition zone diameter of 15 mm, and the 90% honey group had an average of 11.63 mm.

Manuka (Mn) and trigona (Tr) honey were found to minimize the doubling time but not to enhance the number of fibroblast culture cells in a number of earlier experiments, such as those conducted by Priyono et al. (2022). In all Mn and 0.04% Tr groups, there were much more keratinocyte culture cells than in the control group. The quantity of keratinocyte culture cells was positively connected with the length of exposure to Mn 0.04%, 0.1%, and 0.04% Tr. Following exposure to Mn and Tr at three different concentrations—0.04%, 0.1%, and 0.25%—proliferation tests were conducted on fibroblast and keratinocyte cultures obtained from CSOM patients. KGF and bFGF levels were also assessed and compared to the control group.

Tabel 3. Antibacterial activity test of treatment groups against pseudomonas aeruginosa bacteria ATC 27852

Group	Mean ± SD, mm	Median (Min – Max), mm
Control (-)	0	0
Control (+) (Ofloxacin)	30,88 ± 0,63	31 (30 – 31,5)
Honey 60 %	0	0
Honey 70 %	0	0
Honey 80 %	0	0
Honey 90 %	11,63 ± 0,43	11,75 (11 – 12)
Honey 100 %	15 ± 0,54	15,13 (14,25 – 15,5)

Following tympanoplasty, the intervention group experienced a considerably greater percentage of wound drying at weeks 3, 4, and 6 compared to the control group. Through its actions on fibroblasts and keratinocytes, it was determined that administering Mn during tympanoplasty promotes the achievement of flawless MT re-epithelialization and may boost tympanoplasty success. Tr's beneficial effects on fibroblasts and keratinocytes were also demonstrated in this study, allowing for more research into the honey's potential as a treatment.

Consistent with this study, Suwito et al. (2024) investigated the antibacterial properties of Kaliandra and Blora honey against *Staphylococcus aureus (S. aureus* ATCC 25923) and *Escherichia coli* O157:H7 (*E. coli* O157:H7) ATCC 43894. They discovered that the inhibition zone of S. aureus ATCC 25923 in Kaliandra honey occurred at 25% and 50% concentrations, with a diameter of 8.0 ± 0.1 mm and 9.0 ± 0.2 mm. The 50% concentration of *S. aureus* ATCC 25923 in Blora honey resulted in an inhibitory zone that was 7.3 ± 0.1 mm in diameter. At 50% concentration, the inhibition zone of E. coli O157:H7 ATCC 43894 in Kaliandra and Blora honey had diameters of 8.0±0.3 mm and 7.7±0.1 mm, respectively. There was no inhibitory zone for S. aureus ATCC 25923 or E. coli ATCC 43894 in commercial honey at different doses. *S. aureus* and *E. coli* O157:H7 can be inhibited by Kaliandra and Blora honey.

A 2019 study by Yunus et al. sought to determine whether pure forest honey (Meidepuratum) could stop the growth of Staphylococcus aureus germs. It was discovered that the inhibition zone's average diameter was 12.1 mm at a 20% concentration, 13.2 mm at a 20% concentration, 15.6 mm at a 30% concentration, 16 mm at a 40% concentration, and 17 mm at a 50% concentration. These findings suggest that the diameter of the ensuing inhibitory zone will increase with the concentration of forest honey administered.

According to Ellisa et al. (2020), the maximum inhibition zone diameter, 7.88 mm, was at 100% concentration and met the requirements for moderate inhibition. Following a 24-hour incubation period, a solution containing 100% glutathione isolated from S. cerevisiae became transparent. In the meantime, *P. aeruginosa* growth was observed at all concentrations when inoculated into MHA (Muller Hinton Agar) media. The purpose of this study is to demonstrate that glutathione, which was extracted from *S. cerevisiae*, has antibacterial efficacy against *P. aeruginosa* infections. As a result, glutathione derived from *S. cerevisiae* can be utilized as an alternate treatment for *P. aeruginosa* infections because it exhibits antibacterial action against P. aeruginosa.

Tabel 4. Differences in Inhibition Zone Diameter Based on Treatment for the Growth of *Pseudomonas aeruginosa* ATCC 27852 Bacteria

Group	p	Posthoc[b]					
		C (+)	60%	70%	80%	90%	100%
Control (-)	<0,001[a]	0,013	1,000	1,000	1,000	1,000	0,160
Control (+)			0,013	0,013	0,013	1,000	1,000
Honey 60 %				1,000	1,000	1,000	0,160
Honey 70 %					1,000	1,000	0,160
Honey 80 %						1,000	0,160
Honey 90 %							1,000
Honey 100 %							

[a]*Kruskal Wallis,* [b]*Dunn*

Based on the treatment group, a significant difference in inhibition zone diameter was found using the Kruskal-Wallis test ($p < 0.001$). A post-hoc test was used to continue the research and identify whether groups displayed significant variations in inhibitory zone diameter. The positive control group, the 60%, 70%, and 80% honey groups, as well as the negative control group, all had significantly different inhibition zone diameters according to the Dunn test. The inhibition zone diameter did not, however, change significantly between the 90% and 100% honey groups and the positive control group.

Several previous studies, including Hosseini et al. (2024), found that honey significantly reduced the average time required for pain relief and analgesic consumption in the intervention group compared to the control group. This study investigated the effects of locally applied honey on pain intensity, analgesic consumption, pain relief, and nighttime awakenings in children after tonsillectomy. Although honey shows promising results in reducing post-tonsillectomy pain, caution is advised due to the limited quality of evidence. More robust, randomized clinical trials are needed to address bias and strengthen confidence in the findings.

According to Hasanuddin's research from 2023, the effectiveness of forest honey (Apis dorsata) in preventing the growth of Aggregatibacter actinomycetemcomitans bacteria is demonstrated by the diameter of the inhibition zone of these bacteria in forest honey (Apis dorsata) at 80% concentration (13.433 ± 8.936) and at 85% concentration (14.783 ± 5.702). The p-value obtained ($p < 0.01$) indicates a significant difference. We may conclude that

Aggregatibacter actinomycetemcomitans bacteria are effectively inhibited by forest honey (Apis Dorsata) at concentrations of 80% and 85%.

A study conducted in India by Juyal et al. (2017) discovered that 576 (98.8%) of the 583 samples that were gathered showed signs of growth. 187 (32.1%) samples contained isolated *P. aeruginosa* bacteria. The most effective antibiotics were imipenem (70.6%), cefepime (69.0%), amikacin (74.3%), and piperacillin-tazobactam (75.4%). Of the five antibiotic classes investigated, twenty-nine (15.5%) bacteria exhibited resistance. Fluoroquinolones had the highest resistance rate (48.7%), followed by antipseudomonal penicillins (41.7%) and carbapenems (29.4%). The purpose of this study was to discover multidrug-resistant (MDR) P. aeruginosa strains, assess the incidence of P. aeruginosa among patients with CSOM, and examine their patterns of antibiotic susceptibility.

According to Cianciosi et al., (2018), black honey is thought to have various health benefits due to its higher active ingredients. Applying black honey to wounds can reduce inflammation caused by Staphylococcus infections. Black honey, rich in flavonoids and phenol compounds, has been shown to have anti-inflammatory effects. Bacterial infections increase inflammation, slowing wound healing. Conversely, applying black honey to wounds can reduce inflammation, accelerating the healing of wounds infected with Staphylococcus bacteria.

Antibacterial activity test of treatment groups against pseudomonas aeruginosa bacteria ATC 29213

The growth of Staphylococcus aureus ATCC 29213 bacteria was measured in each treatment group. The results are shown in Table 5 together with the mean, standard deviation, median, minimum, and maximum values. According to a study using Staphylococcus aureus ATCC 29213 bacteria, the control group (+) receiving ofloxacin had the highest inhibitory zone diameter, with an average of 30.94 mm, the least being 30.25 mm, and the largest being 31.5 mm. In contrast, an average inhibition zone diameter of 8.63 mm was found in the honey group with a 100% concentration. The average diameter of the inhibitory zone in honey with a 90% concentration was 15.38 mm. Additionally, the average inhibition zone diameter in honey with an 80% concentration was 12 mm.

Table 5. Antibacterial activity test of treatment groups against *Pseudomonas aeruginosa* ATC 29213 bacteria

Group	Mean ± SD, mm	Median (Min – Max), mm
Control (-)	0	0
Control (+) (Ofloxacin)	30,94 ± 0,66	31 (30,25 – 31,5)
Honey 60 %	0	0
Honey 70 %	0	0
Honey 80 %	12 ± 0,54	12,13 (11,25 – 12,5)
Honey 90 %	15,38 ± 0,43	15,25 (15 – 16)
Honey 100 %	18,63 ± 0,52	18,63 (18,63 – 19,25)

According to an Indian study by Kumar et al. (2020), manuka honey seems to be a useful medicinal substance for treating otitis externa. In this study, twenty participants were enrolled. Twenty patients were monitored on the first day, fifteen on the third, and seven on the seventh. There was a statistically significant difference between the average pain score on day 0 (5.35), and the average pain score on day 1 (2.95). After obtaining written informed agreement, patients with acute otitis externa who had no prior interventions or ear complaints were chosen for the trial. Every patient who was chosen for the trial had their umbilical tape wrapped with Manuka honey. On days 0, 1, 3, and 7, pain ratings and canal wall edema were noted. The purpose was to investigate the effectiveness of applying Manuka honey topically to patients suffering from acute otitis externa.

Staphylococcus aureus (48.69%) and *Pseudomonas aeruginosa* (19.89%) were the most frequently isolated causal organisms among 191 aerobic isolates (Prakash et al. 2013). Fungi made up 12.25% of the isolates, whilst anaerobic bacteria made up 29.41%. The aerobic isolates' antimicrobial profile revealed that they were most sensitive to gentamicin (82.7%), ceftriaxone (83.4%), and amikacin (95.5%). The antibacterial activity of honey against the clinical isolate *Pseudomonas aeruginosa* ATCC 27853 was investigated by Mullai and Menon (2005) in India. They discovered that honey may inhibit

Pseudomonas aeruginosa ATCC 27853 with a minimum inhibitory concentration of 11%.

According to Yuliati's (2017) study, which used the well diffusion method to compare the effects of honey solutions on the growth of Pseudomonas aeruginosae and Staphylococcus aureus bacteria at different concentrations: 105 ppm, 106 ppm, 5x106 ppm, 107 ppm, and 108 ppm, the inhibition zone measurement results for Staphylococcus aureus bacteria were classified as weak at 106 ppm. Inhibition zones with average diameters of 6.5 mm, 10.5 mm, 12 mm, and 14.7 mm were produced at 5x106 ppm, 107 ppm, and 108 ppm.

Pseudomonas aeruginosae bacteria were classified as low at concentrations of 106 ppm, 5x106 ppm, and 107 ppm, which produced inhibition zones of 6 mm, 8.5 mm, and 9.5 mm, respectively, and weak at concentrations of 108 ppm, which produced inhibition zones of 10.5 mm. Although the difference is not statistically significant, it may be deduced that the antibacterial efficacy of honey solution is higher against *Staphylococcus aureus* bacteria than against *Pseudomonas aeruginosae* bacteria.

Tarra seeds (A. elasticus) have the potential to be developed as an antibacterial agent, particularly against MDR P. aeruginosa, according to research by Wahdaniar et al. (2023). The purpose of this study is to ascertain whether tarra seed extract (Artocapus Elasticus) has antibacterial activity against Multidrug Resistant (MDR) *Pseudomonas aeruginosa*. The diameter of the inhibition zone as well as the MIC and MBC tests were used to gauge the antibacterial activity test's outcomes. Diffusion test using Agar discs (inhibitory diameter 7-16 mm). The extract's MBC values were 25 and 12.5 mg/mL, while its MIC value was 6.25 mg/mL. Streptococcus mutans bacterial development is effectively inhibited by Jambi Acacia Carpa wild honey, according to studies by Gumilar et al. (2023).

Tabel 6. Differences in Inhibition Zone Diameter Based on Treatment for the Growth of Pseudomonas aeruginosa ATCC 29213 Bacteria

Group	p	Posthoc[b]					
		C (+)	60%	70%	80%	90%	100%
Control (-)	<0,001[a]	0,007	1,000	1,000	1,000	0,664	0,087
Control (+)			0,007	0,007	0,664	1,000	1,000
Honey 60 %				1,000	1,000	0,664	0,087
Honey 70 %					1,000	0,664	0,087
Honey 80 %						1,000	1,000
Honey 90 %							1,000
Honey 100 %							

[a]*Kruskal Wallis,* [b]*Dunn*

The Kruskal Wallis test revealed a significant difference (p <0.001) in the inhibition zone's diameter depending on the treatment group on Staphylococcus aureus ATCC 29213 growth. After that, a follow-up test (post-hoc) was used to continue the study and identify which groups displayed a significant difference in the diameter of the inhibitory zone. The positive control group, the (-) control group, and the 60% and 70% honey groups all had significantly different inhibitory zone diameters, according to the results of the Dunn test. The diameter of the inhibitory zone did not, however, differ significantly between the 80%, 90%, and 100% honey groups and the (+) control group.

This study is consistent with a number of earlier investigations, including one by Lubis et al. (2023), which discovered that the honey group significantly reduced pain on days 1, 2, 4, 7, and 10 ($p = 0.034$; $p = 0.003$; $p < 0.001$; $p = 0.001$; $p = 0.001$) over time, significantly outperforming the placebo and control groups. On average, the honey group's inhibition zone diameter shrank by 4.75 ± 1.28 mm on day 1, 4.13 ± 1.36 mm on day 2, 3.13 ± 0.99 on day 4, 2.00 ± 1.07 on day 7, and 0.88 ± 0.64 on day 10. The same is true for the control and placebo groups. Nonetheless, the honey group saw a quicker decrease in discomfort. The purpose of this study was to assess how honey affected tonsillectomy patients' postoperative discomfort.

Twenty-four adult male patients undergoing tonsillectomy surgery were used in this study. They were split into three groups at random: the honey group, the glucose group, and the control group. regular analgesics and antibiotics were administered to all individuals; the control group received simply the regular postoperative regimen, while the honey group received honey and the placebo group received a placebo. The honey from kapok trees (Ceiba pentandra) was used in this investigation. For ten days, the honey was gargled every six hours. The placebo group was also subjected to the same procedure. A Visual Analogue Scale questionnaire was used to measure pain levels throughout a ten-day period, and on days 1, 2, 4, 7, and 10, the frequency of analgesic drug use was noted. After tonsillectomy, it can be inferred that giving kapok tree honey (Ceiba pentandra) can lessen postoperative pain and the requirement for analgesics. Honey can therefore be regularly used as an extra therapy for individuals recovering from surgery and can be regarded as a complementary treatment.

Only Gram-positive bacteria and specific strains of Gram-negative bacteria were shown to be inhibited in growth by three synthetic disinfectants (Ramzi et al., 2020). NDD9® only demonstrated antibacterial activity against Gram-positive strains of S. aureus and S. aureus ATCC 29213, with a minimum inhibitory concentration (MIC) of 0.25 mg/ml. The disinfectant spray showed effects against all four strains, including *E. coli* (9), *S. aureus, E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853, with an inhibitory concentration of 4 mg/ml. However, the growth of *S. aureus* ATCC 29213 was inhibited at 2 mg/ml. With a MIC of 4 mg/ml, the third disinfectant, Phagosurf ND®, was the only one that inhibited the growth of S. aureus ATCC 29213. This study evaluated the antibacterial activity of three synthetic disinfectants from the quaternary ammonium group against a range of hospital-isolated Gram-positive and Gram-negative bacterial strains, including *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Staphylococcus aureus*. The reference strains were *Pseudomonas aeruginosa* ATCC 27853, which was used as a negative control strain, Escherichia coli ATCC 25922, and Staphylococcus aureus ATCC 29213.

Compared to S. aureus and Enterobacterales, the diameter of the inhibitory zone for *Pseudomonas aeruginosa* was larger, according to research by Cherkaoui et al. (2020). The inhibition zone for each bacteria was *P. aeruginosa* 99.1% (797/804; 95% CI 98.2%–99.6%); *S. aureus* 99.5% (1029/1034; 95% CI 98.9%–99.8%), and Enterobacterales 98.8% (2798/2832; 95% CI 98.3%–99.1%).

Uji AST bakteri *Pseudomonas aeruginosa*

A swab test of a CSOM patient's secretions, which revealed *Pseudomonas aeruginosa* bacteria, using the Vitek 2 compact and AST (Antimicrobial Susceptibility Testing), revealed sensitivity to only Ciprofloxacin, Amikacin, and Levofloxacin. The remaining bacteria were resistant to other antibiotics. The study's findings demonstrated that the inhibition zone's diameter was only discernible in the control group (+) receiving ofloxacin. This group's inhibition zone had an average diameter of 25.5 mm, with the smallest diameter measuring 25 mm and the largest measuring 26 mm. The inhibitory zone diameter was completely absent in the other group. This is in accordance with the results of the AST of *Pseudomonas aeruginosa* bacteria obtained in CSOM patients where it was only sensitive to one antibiotic, Ciprofloxacin (quinolone group), which was also the same as the positive control (+) Ofloxacin.

A significant inhibitory zone ($p = 0.000$) was formed by Sumbawa white honey, according to several earlier experiments by Hazaa et al. (2025). The area of the inhibitory zone was influenced by the honey concentration. The biggest inhibition zone was 3.46 ± 0.72 mm at a concentration of 100%, followed by 0 ± 0 mm at a 25% concentration ($p = 0.003$) and 1 ± 1 mm at a 75% concentration ($p = 0.005$). A higher concentration of Sumbawa NTB white honey would result in a wider inhibition zone. Thus, it can be said that Sumbawa White Honey has antibacterial properties against Pseudomonas aeruginosa, and that the more honey there is in the mixture, the more effective it is. This is seen by the growing inhibition zone size that results from increased honey content. Sumbawa White Honey's efficacy is still inferior to that of chloramphenicol, the positive control, though.

Tabel 7. *Pseudomonas aeruginosa* Bacteria AST Test

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL			Ertapenem		
Ampicillin	0	R	Meropenem	4	I
Ampicillin/ Sulbactam	0	R	Amikacin	8	**S**
Piperacillin/ Tazobactam	>=128	R	Gentamicin		
Cefazolin			Ciprofloxacin	<=0.25	**S**
Ceftazidime	32	R	Tigecycline	>=8	R
Ceftriaxone	0	R	Nitrofurantoin		
Cefepime	>=64	R	Trimepthoprim/ Sulfamethoxazole	0	R
Aztreonam	32	R	Amoxicillin clavulanate	0	R
			Levofloxacin	27	**S**

Description : S : *Susceptible*
I : *Intermediate*
R : *Resistant*

Black honey and forest honey show antibacterial properties against *Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus aureus*, per research by Kaligis et al., (2020). The antibacterial effect of forest honey is slightly greater than that of black honey. Honey contains hydrogen peroxide, antimicrobial proteins, high osmolarity, and an acidic pH, among other antibacterial ingredients. According to the findings of the analysis of the contents of black and forest honey, the former seems to have less sugar, more protein, less hydrogen peroxide, more flavonoids, a higher water content, a more acidic pH, and a higher water content than the latter. According to the honey's antibacterial properties, black honey has a higher water, hydrogen peroxide, and sugar content than forest honey, which has a higher pH, protein, and flavonoid content. His study intends to ascertain the antibacterial activity of black honey and forest honey in vitro against *Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus aureus*.

The Microbiology Laboratory of Sam Ratulangi University's Faculty of Mathematics and Natural Sciences use a dilution method laboratory experiment. 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78% (v/v) of honey were utilized, in that order. According to the study's findings, black honey and forest honey both exhibit antibacterial qualities. Forest honey has a Minimum Inhibitory Concentration (MIC) of 12.5%, 12.5%, and 25% (v/v) against S. aureus, E. coli, and P. aeruginosa, respectively. Black honey's minimum inhibitory concentration (MIC) against *S. aureus, E. coli*, and *P. aeruginosa* was 25%, 12.5%, and 25% (v/v), in that order. Neither black honey nor forest honey had any Minimum Bactericidal Concentration (MBC). Compared to black honey, forest honey has a more potent antibacterial action.

Almasaudi et al., (2016) carried out a study that contrasts the effects of five distinct honey varieties, including imported and local Saudi honey: Manuka Honey UMF +20, Manuka Honey UMF +16, and Active +10. This study is in line with this research. Manuka honey fared better than Sidr honey and Nigella sativa honey when tested against *Staphylococcus aureus*. Both methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* are vulnerable to Manuka Honey UMF +20's bactericidal activities. However, the only action of Sidr honey and N. sativa is bacteriostatic.

The type of honey and the concentration at which it is administered determine how effective different kinds of honey are against *Staphylococcus aureus*. The best bactericidal action is seen in manuka honey. To determine how honey affects bacterial resistance, more research must be done. The effectiveness of wild bee (Apis dorsata) honey against *Staphylococcus aureus* was demonstrated by Dzulasfi (2021) research, which indicated that at a 15% concentration, the average inhibition zone diameter was 22 mm, indicating substantial growth inhibition. The purpose of this study was to evaluate the antibacterial efficacy of honey made from wild bees (*Apis dorsata*) and assess its inhibitory activity against *Staphylococcus aureus* at concentrations of 5% w/v, 10% w/v, and 15% w/v.

Conclusion

This study shows that Indonesian honey has potential as an antibacterial agent against *Pseudomonas aeruginosa* both clinical isolates from Chronic Suppurative Otitis Media (CSOM) patients and the standard strain *P. aeruginosa* ATCC 27852—and *Staphylococcus aureus* ATCC 29213. The antibacterial activity of honey was shown to be concentration-dependent, with the most significant effect observed at honey concentrations ≥90%. The inhibition zone formed showed that honey was able to significantly inhibit the growth of Gram-negative and Gram-positive bacteria, although its inhibitory power was still below that of ofloxacin as a positive control.

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