Original Article

Print ISSN: 2321-6379
Online ISSN: 2321-595X
DOI: 10.17354/ijss/2018/295

The Effect of Garlic (*Allium sativum*) Extract in Suppressing Microbial Growth Isolated from Chronic Suppurative Otitis Media by *In Vitro*

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Abstract

Objective: The objective of the study was to determine the effect of garlic extract in suppressing the microbial growth isolated from chronic suppurative otitis media (CSOM) with various concentrations.

Materials and Methods: This was an experimental study with pretest–posttest design. The extraction of garlic was carried out at Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, by the process of maceration, filtration, and serial dilutions of concentration. Aural discharges isolated from 38 patients with CSOM were cultured for bacterial identification. Afterward, antimicrobial sensitivity test was conventionally performed by dilution and diffusion methods. Dilution test was carried out in determining minimum inhibitory concentration, and the concentration of extracts ranged from 10% to 100%, followed by establishing minimum bactericidal concentration by counting the number of bacterial colonies. Regarding the diffusion test, the bacterial inoculum was grown on agar medium. Antimicrobial disc (garlic extract) was placed on agar surface and allowed to diffuse. Diffusion was started with examining garlic extract at 70%, 80%, 90%, and 100% concentration on the bacteria found in the identification and American Type Culture Collection (ATCC) bacteria with four repetitions. The assessment was evaluated by measuring the clear zone to indicate the extent of the inhibitory zone against bacterial growth.

Results: Bacterial culture examination found seven clinical bacteria and four ATCC bacteria obtaining the largest inhibition zone on *Pseudomonas aeruginosa* ATCC (19.12 mm), followed by clinical *Escherichia coli* (17.75 mm), clinical *P. aeruginosa* (16.25 mm), and *E. coli* ATCC (15.37 mm) at 100% concentration. There were significant mean differences between the zones of inhibition based on the concentrations of extract ($P = 0.007$).

Conclusion: The most significant zone of inhibition of the garlic extract was mainly found on *P. aeruginosa,* and there were significant mean differences between the zones of inhibition at 70% and 80% concentrations and at 80–100% concentrations.

Key words: Chronic suppurative otitis media, Garlic extract, *In vitro*

INTRODUCTION

Chronic suppurative otitis media (CSOM) is one of the diseases in which 94% are in developing countries. In accordance with the criteria of the World Health Organization, Indonesia is categorized as high category with 2–3%.[1]

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Month of Submission : 09-2018
Month of Peer Review : 10-2018
Month of Acceptance : 11-2018
Month of Publishing : 11-2018

The bacteria found in CSOM are different from those found in acute otitis media or otitis media with effusion. The most common bacteria found in CSOM are aerobic bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, Gram-negative bacilli such as *Escherichia coli*, *Proteus* sp., *Klebsiella* sp., and anaerobes *Bacteroides* sp., or a combination of both.[2,3]

However, many CSOM treatments do not use the appropriate antibiotics, thus leading to failure of treatment. Another danger that could arise is the occurrence of microorganism resistance, prolonged infection, and eventually complications.[4]

Antibiotic resistance caused by bacteria has become a serious health problem, and this development occurs due

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to the selection pressures related to the use of antibacterial and the spreading of resistant strain.[5]

The antimicrobial activity of various plants has been studied for years to overcome this resistance issue. This could be useful as an effort to improve the quality of life and avoid the resistance.[6]

Garlic (*Allium sativum*) has the potential as a substitute for antibiotics. The benefits of garlic are numerous, are believed to have antispasmodic, expectorant, antiseptic, bacteriostatic, antiviral, anthelmintic, and antihypertensive activity, and show broad antibiotic properties against Gram-positive and Gram-negative bacteria, including the multiresistant antibiotic strains.[7,8]

MATERIALS AND METHODS

This was an experimental study with pre- and post-design. Data collection was conducted at several hospitals from October 2017 to April 2018. The population met the inclusion criteria: Discharge of old/new CSOM patients was taken from the middle ear/mastoid, patients who were willing to participate, and American Type Culture Collection (ATCC) germs.

In this study, there were seven types of clinical bacteria positive for CSOM and four types of ATCC bacteria with 4 times replication.

The garlic extract was made at the Faculty of Mathematics and Natural Sciences, Organic Chemistry, Universitas Sumatera Utara, Medan by weighing, macerating, filtration use buchner then refiltration of maceration 3 times where the extract was considered 100%.

The ear discharge was taken with swab Amies for the culture and bacterial identification followed with conventional antimicrobial sensitivity test with dilution and diffusion methods. The dilution test was done to determine minimum inhibitory concentration followed by minimum bactericidal concentration determination to calculate the number of bacterial colonies.

The diffusion tests were conducted using diffusion disc method. In this method, the bacterial inoculum was planted in the agar medium. Antimicrobial discs were placed on the agar surface and allowed to diffuse into the surrounding media. Then, the antimicrobial inhibition zone was observed for bacterial growth. The size of the clear zone depends on the speed of diffusion of antimicrobials, the degree of sensitivity of microorganisms, and the rate of bacterial growth. The diffusion began by testing the concentration of garlic extract 70%, 80%, 90%, and 100% on the types of bacteria found from CSOM discharge and ATCC bacteria such as *P. aeruginosa* 27853, *S. aureus* 29213, *E. coli* 25922, and *Klebsiella pneumoniae* 43816 with four repetitions.

Data processing was done using Statistical Package for the Social Sciences with one-way analysis of variance if the distribution was normal and with Kruskal–Wallis test if the distribution was not normal. This study had been approved by the Research Ethics Committee of the Faculty of Medicine, University of North Sumatra.

RESULTS

Table 1 shows that culture examination was found the bacteria that cause CSOM as many as seven types of bacteria.

From Table 2 at a concentration of 70%, the largest inhibitory zone was found in *E. coli* clinics with a mean inhibitory zone of 12.12 mm followed by *P. aeruginosa* ATCC bacteria with a mean inhibitory zone of 10.25 mm. The inhibitory zone was not formed for four types of bacteria, namely *E. coli* ATCC, *Proteus vulgaris, K. pneumoniae, and Proteus mirabilis.*

From Tabel 3 we found at a concentration of 80% the *Escherichia coli* inhibition zone increased to 14.87 mm, as

Table 1: Type of bacteria

Bacteria
Pseudomonas aeruginosa
Escherichia coli
Proteus vulgaris
Klebsiella pneumoniae
Proteus mirabilis
Enterobacter aerogenes
Staphylococcus aureus

Table 2: Inhibition zone based on bacteria type at 70% concentration

Type of bacteria	Repeat				Mean
	I	II	III	IV	
P. aeruginosa ATCC	10.5	10	10	10.5	10.25
P. aeruginosa	9	8.5	9	8.5	8.75
E. coli ATCC	0	0	0	0	0
E. coli	12	12	12.5	12	12.12
P. vulgaris	0	0	0	0	0
K. pneumoniae ATCC	0	0	0	0	0
K. pneumoniae	0	0	0	0	0
P. mirabilis	0	0	0	0	0
E. aerogenes	8,5	8	8.5	8.5	8.37
S. aureus ATCC	9.5	9	9.5	9	9.25
S. aureus	9	9.5	9.5	9	9.25

ATCC: American Type Culture Collection, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *P. mirabilis*: *Proteus mirabilis*, *E. aerogenes*: *Enterobacter aerogenes*, *S. aureus*: *Staphylococcus aureus*, *K. pneumoniae*: *Klebsiella pneumoniae* well as the *Pseudomonas aeruginosa* ATCC inhibition zone to 14.75 mm

Table 3: Inhibition zone based on bacteria type at 80% concentration

Type of bacteria	Repetition				Mean
	I	II	III	IV	
P. aeruginosa ATCC	15	14.5	14.5	15	14.75
P. aeruginosa	12	12	11.5	11	11.62
E. coli ATCC	9	8.5	8.5	8.5	8.62
E. coli	15	14.5	15	15	14.87
P. vulgaris	0	0	0	0	0
K. pneumoniae ATCC	9	8.5	8.5	8	8,49
K. pneumoniae	9	8.5	9	8.5	8.75
P. mirabilis	9	8.5	9	8.5	8.75
E. aerogenes	9.5	9	9.5	9.5	9.37
S. aureus ATCC	11	10.5	10.5	10	10.49
S. aureus	12	11.5	12	11.5	11.75

ATCC: American Type Culture Collection, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *P. mirabilis*: *Proteus mirabilis*, *E. aerogenes*: *Enterobacter aerogenes*, *S. aureus*: *Staphylococcus aureus*, *K. pneumoniae*: *Klebsiella pneumoniae*

Table 4: Inhibition zone based on bacteria type at 90% concentration

Type of bacteria	Repeat				Mean
	I	II	III	IV	
P. aeruginosa ATCC	17	17	16	16	16.49
P. aeruginosa	14	13,5	14	13.5	13.75
E. coli ATCC	12	12	11.5	12	11.87
E. coli	16	16	16	17.5	16.36
P. vulgaris	0	0	0	0	0
K. pneumoniae ATCC	10	9	10	9.5	9.62
K. pneumoniae	11	10	10	10	10.24
P. mirabilis	12	11	11	10.5	11.11
E. aerogenes	11	10.5	11	11	10,87
S. aureus ATCC	12	11.5	12	11.5	11.75
S. aureus	14	14	13.5	13.5	13.75

ATCC: American Type Culture Collection, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *P. mirabilis*: *Proteus mirabilis*, *E. aerogenes*: *Enterobacter aerogenes*, *S. aureus*: *Staphylococcus aureus*, *K. pneumoniae*: *Klebsiella pneumoniae*

Table 5: Inhibition zone based on bacteria type at 100% concentration

Type of bacteria	Repetition				Mean
	I	II	III	IV	
P. aeruginosa ATCC	19.5	18.5	19	19.5	19.12
P. aeruginosa	16.5	16	16.5	16	16.25
E. coli ATCC	15.5	15.5	15	15.5	15.37
E. coli	18	17.5	17.5	18	17.75
P. vulgaris	0	0	0	0	0
K. pneumoniae ATCC	12	11.5	11.5	11	11.50
K. pneumoniae	13	12.5	12.5	12.5	12.62
P. mirabilis	15	14.5	15	15	14.87
E. aerogenes	11.5	11	12	11.5	11.50
S. aureus ATCC	13.5	13	13	12.5	13
S. aureus	16	15.5	16	16.5	16

ATCC: American Type Culture Collection, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *P. mirabilis*: *Proteus mirabilis*, *E. aerogenes*: *Enterobacter aerogenes*, *S. aureus*: *Staphylococcus aureus*, *K. pneumoniae*: *Klebsiella pneumoniae*

From Table 4 at the concentration of 90%, the largest inhibition zone average was shown by *P. aeruginosa* ATCC at 16.49 mm.

-We can see from table 5 at the concentration of 100%, the largest inhibition zone average was shown by *P. aeruginosa* ATCC at 19.12 mm followed by Escherichia coli Clinic at 17.75 mm. From a concentration garlic extract of 70% -100% no inhibition zone was found in the Proteus vulgaris bacteria.. The smallest inhibitory zone was shown by *K. pneumoniae* ATCC and *Enterobacter aerogenes* bacteria with a mean inhibitory zone of 11.50 mm.

Figure 1 show that from concentration 70% the number of bacterial colonies has experienced reduction and concentration 80%, 90% no bacterial growth was found.

Figures 2 and 3 show that the antimicrobial activity of garlic extract (*Allium sativum*) using disc diffusion method shows the extent of the inhibitory zone of garlic extract at each concentration starting from 70%, 80%, 90%, to 100% with four repetitions.

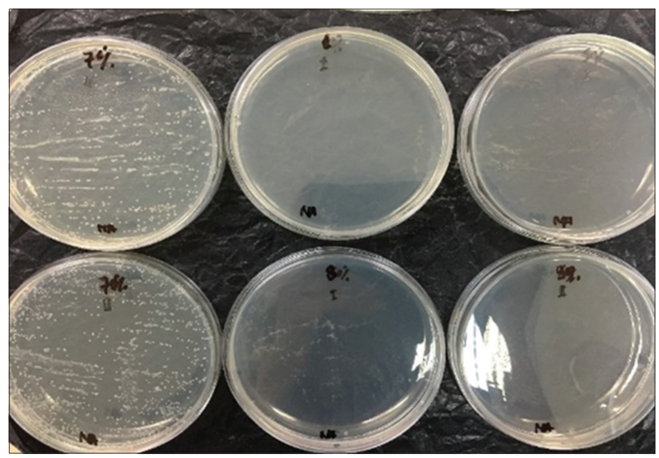

Figure 1: Concentration 70% the number of bacterial colonies has experienced reduction and concentrations 80%, 90%, and 100% no bacterial growth was found

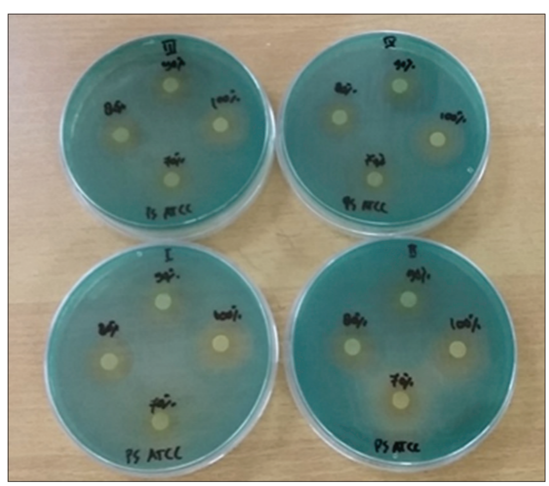

Figure 2: American type culture collection *Pseudomonas aeruginosa*

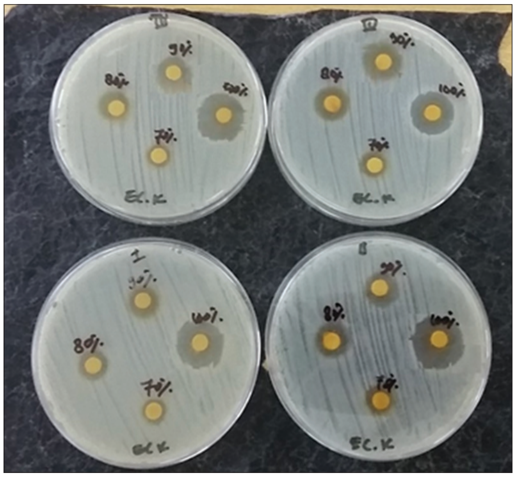

Figure 3: Clinical *Escherichia coli*

Table 6: Inhibition zones from various concentrations based on repetition I to IV for all bacteria

Concentration of garlic extract	Inhibitory zone, mean (SD), mm repetition			
	I	II	III	IV
70%	5.32 (5.17)	5.18 (5.06)	5.36 (5.23)	5.23 (5.10)
80%	9.86 (3.93)	9.64 (3.94)	9.82 (3.98)	9.59 (4.03)
90%	11.59 (4.47)	11.32 (4.50)	11.36 (4.34)	11.36 (4.52)
100%	13.68 (4.47)	13.23 (4.99)	13.46 (5.06)	13.46 (5.22)

SD: Standard device

Table 7: The differences of the mean inhibitory zones from various concentrations

Concentration of garlic extract	Inhibitory zone, mean (SD), mm	*P**
70%	5.27 (0.08)	0.007
80%	9.73 (0.13)	
90%	11.41 (0.12)	
100%	13.46 (0.18)	

*Kruskal–Wallis, SD: Standard device

Table 6 presents the mean inhibitory zone for all bacteria examined for replication I to replication IV. The highest mean inhibitory zone is shown by extract with a concentration of 100%. The results of the study showed that the increase in extract concentration further expands the inhibitory zone with 5.27 mm at 70% concentration up to 13.46 mm at 100% concentration.

We used the Kruskal Wallis test, From the Table 7 it was shown that there was a significant difference in the mean inhibitory zone based on the extract concentration (p = 0.007). The results of the further analysis [Table 3] showed that there were significant differences in mean inhibitory zones between extracts with 70% concentration and 80–100%. Similarly, between 80% and 100% concentrations, a significant difference was also found.

DISCUSSION

In this study, it was found that the bacteria which cause the CSOM consist of *P. aeruginosa, E. coli, P. vulgaris, K. pneumoniae, P. mirabilis, E. aerogenes,* and *S. aureus.*

A similar finding was found in the study by Rath *et al.*, in India. From 1230 samples, it was obtained that 1164 samples had bacterial and fungal growth. The most common bacteria were *P. aeruginosa* 371 isolates, *S. aureus* 220 isolates, *E. coli* 98 isolates, *Klebsiella* sp. 92 isolates, *Proteus* sp. 60 isolates, and *Enterobacter* sp. 19 isolates. This is different from this current study.[9] Ahmad in Saudi Arabia found that the microorganisms that cause CSOM are *S. aureus, P. aeruginosa, P. mirabilis, E. coli,* and *Citrobacter* spp.[10] Both the Gram-negative and Gram-positive bacteria can infect the middle ear. However, in general, more cases were caused by Gram-negative bacteria, as *Pseudomonas* is more durable to survive and produce pyocyanin and bacteriocins and encased by exopolysaccharides.[11]

There were differences in inhibitory zones in each type of bacteria with different concentrations. In this study, the most extensive inhibitory zone was found in *P. aeruginosa* bacteria at a concentration of 100% with a mean of 19.22 mm followed by *E. coli* with a mean of 17.75 mm and *S. aureus* with a mean of 16.5 mm. This result could be found in a study by Metwali *et al.*, on 1:1 garlic oil to Mueller-Hinton agar with an inhibitory zone of 24 mm of *E. coli* and 17 mm of *S. aureus*. In contrast to a study by Verma *et al.* (2015), at a concentration of 100%, the inhibitory zone of *S. aureus* was wider than *E. coli* of 21 mm and 16 mm, respectively. For *P. aeruginosa*, it was found that it has the broadest inhibitory zone of 19.22 mm compared to *E. coli* with a mean of 17.75 mm and *S. aureus* of 16.5 mm.[12]

CONCLUSION

The most significant zone of inhibition of the garlic extract was mainly found on *P. aeruginosa*. Garlic extract can inhibit bacterial growth by finding an inhibitory zone produced. The higher the concentration of garlic extract, the wider the inhibitory zone produced.

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How to cite this article: Ginting R, Zahara D, Aboet A. The Effect of Garlic (*Allium sativum*) Extract in Suppressing Microbial Growth Isolated from Chronic Suppurative Otitis Media by *In Vitro*. Int J Sci Stud 2018;6(7):1-5.

Source of Support: Nil, **Conflict of Interest:** None declared.