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The Antibacterial Activity of *Thermoactinomyces* sp. (H24) Extract Against *Escherichia coli* and *Staphylococcus aureus*

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Abstract

Bacteria of the genus *Thermoactinomyces* have the ability to produce antibacterial bioactive compounds. This bioactive compound can be used for combating diarrheal agents such as *Escherichia coli* and *Staphylococcus aureus.* This study aims to determine the antibacterial activity of the metabolite extract from *Thermoactinomyces* sp. (H24) against *E. coli* and *S. aureus.* Methanol was used as a solvent for the extraction of bacterial bioactive compounds. Antibacterial activity was analyzed by the diffusion method with several extract concentrations (0.75 mL, 1.5 mL, 2.25 mL, and 3 mL), 10% DMSO (Dimethyl sulfoxide) as the negative control, and ciprofloxacin as the positive control. Our result shows that *Termoactinomyces* sp. (H24) extract has an inhibitory effect on the growth of *E. coli* and *S. aureus* with an effective concentration of 2.25 mL (inhibition strength: very strong).

Keywords

Antibacterial, *Escherichia coli*, *Staphylococcus aureus*, *Thermoactinomyces* sp. (H24).

INTRODUCTION

Eschericia coli and *Staphylococcus aureus* are two common causative agents of serious infections in humans (1,2). *E. coli* can infect the respiratory and digestive tracts and cause several diseases like pneumonia, endocarditis, wound infection, meningitis (3), and diarrhea by producing enterotoxins (4). *S. aureus* can cause several diseases such as pneumonia, osteomyelitis, arthritis, and inflammation of the brain. *S. aureus* cause hemolysis of blood, and have the ability to

produce enterotoxins that led to diarrhea, seizures, and fever (5).

The emergence of various kinds of diseases caused by pathogenic bacteria encourages the researcher to find alternative antibacterial compounds. *Thermoactinomyces* sp. (H24) is one of the bacteria with the ability to produce the antibacterial compound (6) . Jawetz *et al.* (7) find that *Thermoactinomyces* sp. (H24) have antifungal activity.

Thermoactinomyces sp., in addition to antifungal compound, also produce antibacterial aromatic compound namely Cyclic Hexapeptides thermoactinoamide A-F with ability to inhibit lipophilic bacteria (8). Currently, there is no reported research on the antibacterial analysis of *Thermoactinomyces* sp. (H24) for inhibiting the growth of several pathogenic bacteria such as *E.coli* and *S. aureus*. Therefore, this research was conducted to analyze the antibacterial activity of *Thermoactinomyces* sp. (H24) for inhibiting the growth of *S. aureus* and *E. coli.*

MATERIALS AND METHODS

Preparation and Sterilization

The media used were Nutrient Agar (NA), Mueller Hinton Agar (MHA), and Nutrient Broth (NB) dissolved in 1,000 mL of distilled water. All media, Petri dishes, and test tubes were sterilized using an autoclave at 121°C, 2 atm for 15 minutes (9).

Extraction of *Termoactinomyces* sp. (H24) Metabolites

Thermoactinomyces sp. (H24) was cultivated by the streak plate method on Nutrient Agar, then incubated at 50°C for 24 – 72 hours. A single colony of *Thermoactinomyces* sp. (H24) was inoculated on 50 mL of Nutrient Broth, then incubated in an incubator shaker at 110 rpm, 50°C for 84 hours until the culture reached a stationary phase. *Thermoactinomyces* sp. (H24) metabolite was extracted by centrifuging the culture at 3,000 rpm for 15 minutes to obtain supernatant. The supernatant obtained was a source of secondary metabolites (9). The supernatant was filtered using Whatman paper no. 1. The filtrate was immersed in 100% methanol for 24 hours in different bottles with a ratio of v/v (1:1). The solvent was removed using a rotary evaporator and then allow to stand for 24 hours (9).

Qualitative Test of *Thermoactinomyces* sp. (H24) Extract

A qualitative test was done to analyze the presence of phenols, alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids in *Thermoactinomyces* sp. (H24) extract.

Preparation of *Escherichia coli* and *Staphylococcus aureus* culture

The colony of *E. coli* and *S. aureus* was transferred into 9 mL of sterile distilled water until the Optical Density (OD) value similar to 0.5 Mc Farland Standard. McFarland

Standard was prepared by mixing H_2SO_4 with 0.05 ml of BaCl 1% in a test tube (10).

Antibacterial Activity Test of *Thermoactinomyces* sp. (H24) Extract

Various concentration of *Thermoactinomyces* sp. (H24) extract (0.75 mL, 1.5 mL, 2.25 mL, and 3 mL) was transferred into 3 ml sterile distilled water. Antibacterial activity test was performed by well diffusion method. *E. coli* and *S. aureus* culture were swabbed evenly on the all surface of the MHA medium using a sterile cotton swab. The well was made on a medium with a diameter of 6 mm, then filled with 25 µL of each extract with several concentrations, DMSO 10% as a negative control, and ciprofloxacin 0.25 µg/mL as a positive control. The medium was incubated at 37° C for 24 – 48 hours (11). A clear zone formed after incubation was measured using a caliper. The clear zone categorized into very strong (diameters >20 mm); strong (diameter 11 – 20 mm); moderate (diameter 6 – 10 mm); and weak (<5 mm) (12).

RESULTS

Qualitative Test of *Thermoactinomyces* sp. (H24) Extract

The qualitative test was revealed the presence or absence of secondary metabolites from *Thermoactinomyces* sp. (H24) extract (Table 1).

Table 1. Qualitative test results of *Thermoactinomyces* sp. (H24) extract

No.	Secondary Metabolites	Reactor	Change	Result
1.	Alkaloids	Wagner	Brown deposits are formed	+
2.	Flavonoids	Magnesium (Mg)andamyl-alkohol	No yellow discoloration occurs	-
3.	Saponins	HCl	No foam	-
4.	Tannins	$FeCl_3$	No dark blue discoloration	-
5.	Phenol	Etanoland $FeCl_3$	No change into green color	-
6.	SteroidsandTerpenoids	CH3COOH glacial, H_2SO_4	No change into red or green	-

Note:
+ : contains secondary metabolites;
– : not contains secondary metabolites

A qualitative test of the *Thermoactinomyces* sp. (H24) extract showed the presence of the alkaloid compounds in the extract, indicated by the formation of brown deposits when Wagner's reagent was added to the extract.

Antibacterial Activity of *Thermoactinomyces* sp. (H24)

The formation of the clear zone indicated the ability of *Thermoactinomyces* sp. (H24) extract to inhibit the growth of E. coli and S. aureus. The diameter of the clear zone showed that the extract had various bacterial growth inhibition level against E. coli from moderate to very strong depending on extract concentration (Table 2). Statistics analysis showed that the different concentrations give significantly different results.

Table 2. Average diameter of clear zone against *Escherichia coli*

Concentration of extract (mL)	Diameter (mm)		Category
	24 hrs	48 hrs	
DMSO 10%	0.00	0.00	-
0.75	9.44	9.62	Moderate
1.5	16.18	16.71	Strong
2.25	21.78	28.49	Very strong
3	26.85	32.94	Very strong
Ciprofloaxcin	37.57	41.20	Very strong

Figure 1 showed the clear zone formed after incubation 24 hours and 48 hours, indicated the ability of *Thermoactinomyces* sp. (H24) extract against *E. coli*.

Figure 2 showed the clear zone formed after incubation 24 hours and 48 hours, indicated the ability of *Thermoactinomyces* sp. (H24) extract against *S. aureus*.

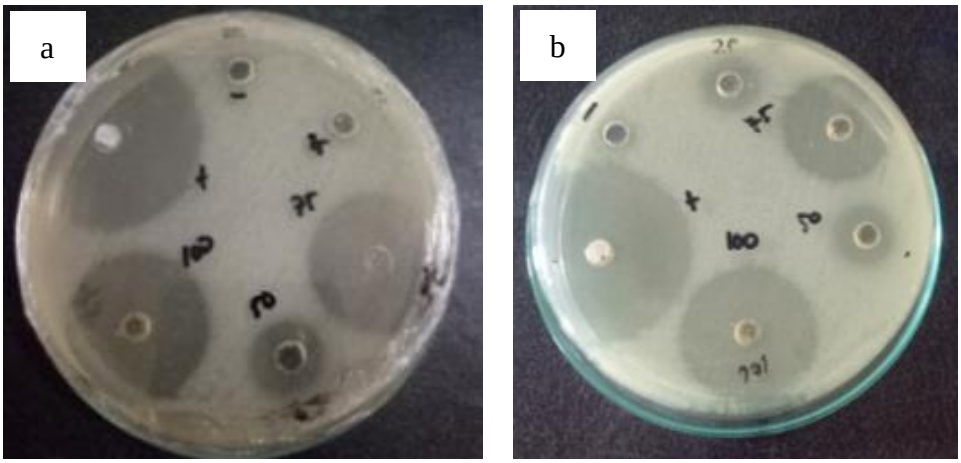

Figure 1. The clear zone of *Thermoactinomyces* sp. (H24) extract against *Escherichia coli*: (a) 24 hours, (b) 48 hours of incubation

Table 3. Average diameter of clear zone against *Staphylococcus aureus*

Concentrationof Extract (mL)	Diameter (mm)		Category
	24 hrs	48 hrs	
DMSO 10%	0.00	0.00	-
0.75	7.41	7.43	Moderate
1.5	13.52	13.71	Strong
2.25	22.31	22.68	Very strong
3	26.12	36.48	Very strong
Ciprofloaxcin	36.16	36.48	Very strong

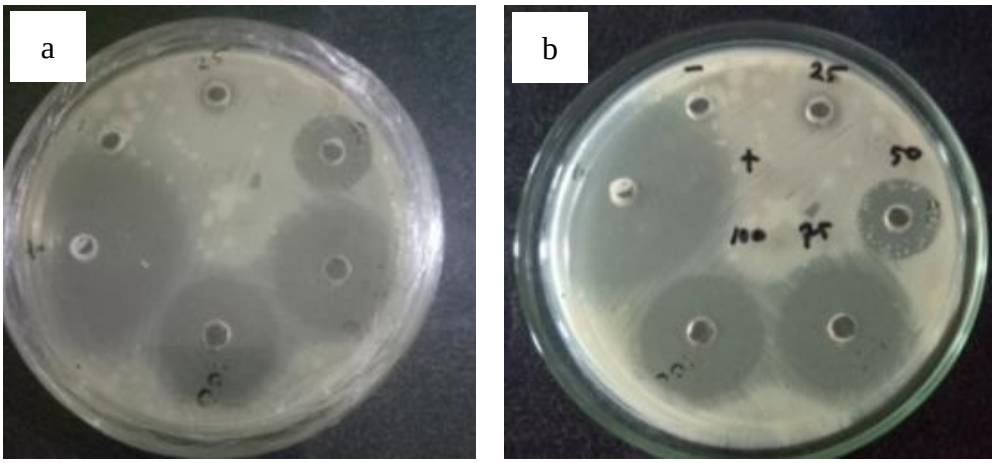

Figure 2. The clear zone of *Thermoactinomyces* sp. (H24) extract against *Staphylococcus aureus*: (a) 24 hours, (b) 48 hours of incubation

DISCUSSION

Thermoactinomyces sp. (H24) extract has the ability to inhibit the growth of *E. coli* and *S. aureus*, indicated by the formation of a clear zone around the well. The effective concentration of *Thermoactinomyces* sp. (H24) extract against *E. coli* and *S. aureus* was 0.75 mL, 1.5 mL, 2.25 mL, and 3 mL with inhibition strength from moderate to very strong.

Thermoactinomyces sp. (H24) extract concentration of 0.75 mL could inhibit *E. coli* and *S. aureus* at a moderate level (Table 2 and Table 3). Teta *et al.* (8) was reported that *Thermoactinomyces vulgaris* isolated from a hydrothermal vent on the coast of Iceland possessed moderate level antibacterial activity with a concentration of at least 0.35 mL against *S. aureus* and 0.14 ml against Escherichia coli. Bratchkova *et al.* (13) showed that *Thermoactinomyces* sp. isolated from penguin droppings on Livingston Island, Antarctica had a strong level of antibacterial activity at the volume of 0.10 mL against *S. aureus* and *E. coli*. This high antibacterial activity at a low concentration from *Thermoactinomyces* sp. extract reported by Teta *et al.* (8) and Bratchkova *et al.* (13) because of the different extraction solvents

and the use of chromatography result in the more pure extract.

The concentration of extract positively correlated with the diameter of the clear zone. The clear zone formed is produced from the antibacterial active substance in the extract. The most effective concentration of extract for inhibiting the growth of *E. coli* and *S. aureus* is 2.25 mL. *E. coli* cell walls have three layers (multilayer) with high-fat content (11 – 22%) and peptidoglycan layer inside stiff layer (10% dry weight) that cause *E. coli* less absorb antibacterial compound (14). The effective concentration of antibacterial extract produces a biological response. The effective concentration is defined as the lowest concentration that provides a significant response (15). The diameter of the inhibition zone at the 48-hours incubation time was greater than 24-hours incubation, indicated that the antibacterial activity is bactericidal or has the ability to kill bacteria (16).

The average diameter of the inhibition zone resulted from the *Thermoactinomyces* sp. (H24) extract against *E. coli* was greater than *S. aureus*. This difference indicates that Gram-negative and Gram-positive bacteria have different responses to antibacterial compounds. *E. coli*, a Gram-negative bacteria, is more sensitive to non-polar (hydrophobic) antibacterial compounds such as alkaloid compounds than Gram-positive bacteria such as *S. aureus* (20). The difference in response between Gram-positive and Gram-negative bacteria to alkaloid compounds is due to the cell walls of Gram-negative bacteria contain 11–22% lipids which will more easily react with nonpolar alkaloid compounds, resulting in cell lysis. In contrast, Gram-positive bacteria, such as *S. aureus*, contains 4% lipids which make it difficult to react with alkaloid compounds. Bratchkova *et al.* (13) reported that *E. coli* (Gram-negative) was more sensitive than *S. aureus* (Gram-positive) bacteria in response to *Thermoactinomyces* sp. Impact-14 extract.

The antibacterial activity of *Thermoactinomyces* sp. (H24) extract against *E. coli* and *S. aureus* is due to the content of metabolite compounds produced by *Thermoactinomyces* sp. (H24). The qualitative test showed the presence of alkaloid compounds in *Thermoactinomyces* sp. (H24) extract (Table 1). Several studies also show that many Thermoactinomyces strains extract contained alkaloid compounds as antibacterial agents. Bratchkova *et al.* (13) explained that *Thermoactinomyces* sp. impact-14 isolated from penguin droppings on Livingston Island, Antarctica contains β-carboline alkaloid compounds which have antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus mycoides*, *Streptomyces viridoch*, *Proteus vulgaris*, *Candida*

tropicalis, *Candida albicans*, *Mucor miehei*, and *Penicillium notatum*.

Alkaloid compounds found in the *Thermoactinomyces* sp. (H24) extract play a role in inhibiting the growth of *E. coli* and *S. aureus*. The nitrogen group in the alkaloid will react with the amino acids that cause the change in the structure of the amino acid in the bacterial cell wall. Changes in the structure of amino acids trigger lysis in bacterial cells led to bacterial death (17).

CONCLUSIONS

Thermoactinomyces sp. (H24) extract contained an alkaloid compound and has antibacterial activity against *E. coli* and *S. aureus*. Strong inhibition activity against *E. coli* and *S. aureus* was achieved by the concentration of 50% *Thermoactinomyces* sp. (H24) extract.

AUTHOR CONTRIBUTIONS

Julia Nanda Puspita: conceptualization, methodology, formal analysis, investigation, data curation. Rahmawati: validation, writing-review & editing, project administration, funding acquisition. Rikhsan Kurniatuhadi: validation, writing-original draft, project administration.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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