Antibacterial Effect of Methanol Extracts from Edible Rhizomes Against *Salmonella typhi* ATCC-422

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Abstract. This research was conducted to determine the antibacterial effect of methanol extracts of four edible rhizomes namely temu putih *(Curcuma zedoaria),* temu ireng *(Curcuma aeruginosis)*, temu giring *(Curcuma heyneana)* and temu kunci *(Boesenbergia pandurata).* The extracts of temu putih, temu ireng, temu giring and temu kunci were obtained by maceration method using methanol. The antibacterial activity test was carried out using the modified agar diffusion method against *Salmonella typhi* ATCC 422. With 1% of the four methanol extracts showed that methanol extract of temu kunci have the highest antibacterial activity against *S. typhi* with an inhibition zone diameter of 20.3 mm and a minimum inhibitory concentration value of 0.0078%.

Keywords: Antibacterial · Concentration · Edible rhizomes · Minimum Inhibitory

1 Introduction

Excessive use of antibiotics has led to an increase in cases of antibiotic resistance. Treatment from natural ingredients is needed to be an alternative to cure infectious diseases so that they can reduce the occurrence of antibiotic resistance and have potential as antibacterial. Rhizome is a part of plants that has been known as kitchen ingredients and also used as traditional medicine by the local people in Indonesia to prevent various diseases caused by infection with microorganisms. Utilization of the rhizome of temu putih, temu kunci, temu giring and temu ireng can be a source of constituents that are nutritious for the health of the body.

Salmonella typhi is a bacterial pathogen that causes typhoid fever, an infectious disease with prolonged fever with inflammation that can damage the intestines and liver. S. typhi is a Gram-negative rod that does not have spores, moves with peritrichous flagella, is facultative intracellular, and is facultatively anaerobic. Varies in size 0.7–1.5 × 2–5 m, has somatic antigen (O), flagellar antigen (H) with two phases, and capsular antigen (Vi). These bacteria are resistant to selenite and sodium deoxycholate, which can kill other bacteria by producing endotoxins, invasion proteins and MRHA (Mannose

T. D. Larasati et al. (Eds.): ICTROPS 2022, ABSR 31, pp. 24–30, 2023.
https://doi.org/10.2991/978-94-6463-180-7_5

Resistant Hemagglutinin). *S. typhi* can survive for several months to a year when present in feces, butter, milk, cheese, and frozen water. *S. typhi* is an obligate intracellular parasite that can live in macrophages and cause gastrointestinal symptoms only late in the illness, usually after a fever. Prolongation, bacteremia, and finally localization of infection in the submucosal lymphoid tissue of the small intestine [1].

According to [2], white turmeric can be used as anticancer, antibacterial, antithrombic, antifungal, antioxidant and hepatoprotective. Temu Kunci contains essential oils that can be used as antimicrobials and several useful compounds as anticancer and antioxidants [3]. Temu giring has bioactivity as antiaging, antioxidant and anti-inflammatory [4]. Temu ireng is known to contain flavonoids that can function as antioxidants and antimicrobials [5].

Based on this description, it is necessary to conduct research on the antibacterial activity of the rhizome temu putih (Curcuma zedoaria), temu kunci (Boesenbergia pandurata), temu giring (Curcuma heyneana) and temu ireng (Curcuma aeruginosis) against Salmonella typhi ATCC 422 test bacteria.

2 Methods

The research was carried out at the Laboratory of Biochemistry and Organic Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan.

2.1 Research Procedure

2.1.1 Plant Material and Extraction

With 1 kg of the dried rhizomes of temu putih, temu ireng, temu giring and temu kunci were collected from Samarinda, East Kalimantan, Indonesia and extracted by with methanol by maceration method. Each sample was filtered and evaporated under reduced pressure to obtain temu putih, temu ireng, temu giring and temu kunci extracts. Those extracts were screening on antibacterial activity against *Salmonella typhi* ATCC 442.

2.1.2 Antibacterial Strains and Culture of Test

This study was used bacteria strain of Salmonella typhi ATCC 442 for antibacterial activity test. The bacteria was activated by using nutrient agar (NA) and nutrient broth (NB).

2.1.3 Screening of Antibacterial Activity

Screening of antibacterial activity of the rhizome of temu putih (Curcuma zedoaria), temu ireng (Curcuma aeruginosa), temu giring (Curcuma heyneana) and temu kunci (Boesenbergia pandurata) against tested bacteria was carried out using the modified agar well diffusion method [5–8]. (Nutrient broth was inoculated with tested bacteria and incubated at 37 °C for 18 to 24 h [4].

By 10 mL of NA (Nutrient Agar) was poured into a sterile petri dish. The required number of wells were cut using a sterile cork borer ensuring proper distribution of wells. After that, the bacterial inoculum was uniformly spread on a sterile petri dish nutrient agar. With 1% of each extract was poured into the well and incubated for 18–24 h at 37 °C. The experiment was performed in triplicate and average zone of inhibition was calculated. It was also carried out with the same experiment for positive (Chloramphenicol) and negative control (methanol).

2.1.4 Determination of MIC (Minimum Inhibitors Concentration) Value

MIC value of rhizome extract which has the highest inhibition zone diameter in the screening test was selected and performed by the same method for screening test. Furthermore, various concentrations of the methanol extract were prepared. This experiment was also carried out for positive and negative controls. After that, it was incubated for 18–24 h at 37 °C. Then, the antibacterial effect were calculated by measuring the diameter of inhibition zone around the well. The MIC value was determined based on the smallest concentration of the sample that still has antibacterial activity as indicated by the formation of an inhibition zone around the well.

3 Results and Discussion

3.1 Extraction

The extraction process of the rhizomes of temu putih (Curcuma zedoaria), temu kunci (Boesenbergia pandurata), temu giring (Curcuma heyneana) and temu ireng (Curcuma aeruginosis) were carried out using the maceration method which had previously been dried without exposure to sunlight for ±4 weeks. The drying process was carried out to remove water content and prevent fungal growth on the rhizome samples. The maceration method is a separation method that uses simple equipment, at room temperature which allows a lot of extracted compounds and the active substances contained in the sample will not be damaged [9]. The dry samples of temu ireng, temu giring, temu putih and temu kunci were extracted using methanol to yield temu putih (38 g), temu ireng (26 g), temu giring (112 g) and temu kunci (110 g) extracts. Those extracts at 1% were screening n antibacterial activity against five pathogenic bacteria. The yield of rhizome methanol extract after rotary evaporator is as follows (Table 1):

Table 1. The Yield of Methanol Extract Rhizomes.

Methanol Extract	Mass (g)	Yield (%)
Temu Putih	38	3,49
Temu Ireng	26	2,66
Temu Giring	112	14,00
Temu Kunci	110	11,49

3.2 Screening of Antibacterial Activity

Screening of antibacterial activity of the methanol extracts of the rhizome temu putih, temu kunci, temu giring and temu ireng at a concentration of 1% against *S. typhi* bacteria using the agar diffusion method with modified wells. According to [10], this method is easier to measure the formation of the inhibition zone area because bacterial activity can reach all layers of nutrient agar.

The antibacterial power can be categorized based on the diameter of the inhibition zone formed which is divided into excellent (inhibition zone more than 15 mm), very good (inhibition zone 13.1–15.0 mm), good (inhibition zone 10.1–13.0 mm), moderate (inhibition zone 8.1–10.0 mm), weak (inhibition zone 6.1–8.0 mm) and inactive (inhibition zone less than or equal to 6 mm) [11].

The results of the screening test for antibacterial activity on the methanol extract of four rhizomes against *S. typhi* bacteria can be seen in Table 2.

Based on Table 2, the inhibition of *S. typhi* was shown by the methanol extract of temu kunci and temu giring with excellent activity through diameter of inhibition zones of 20.3 and 19.00 mm and temu Ireng and temu putih performed good activity with inhibition zones of 12.7 and 10.7 mm, respectively. The presence of secondary metabolites in those methanol is indicated to be the key of their high inhibitory effect toward S. typhi [12] reported that temu kunci rhizome contains secondary metabolites of terpenoids, flavonoids and essential oils while Temu putih contains flavonoids, polyphenols and triterpenoids. In addition, temu giring also contains phenolic and flavonoid compounds [4]. Based on the research of [13], secondary metabolites including flavonoids, alkaloids, phenolics, saponins, tannins and triterpenoids were recorded. Apriyuslim et al. [14], reported that alkaloids have antibacterial activity by damaging cell walls through the constituent components of peptidoglycan in bacterial cells while flavonoids and phenolic derivatives work by denaturing and coagulating bacterial cell proteins. Tannins also have antibacterial activity by means of protein precipitation, enzyme inactivation, and destruction or inactivation of genetic material. Lipophilic terpenoids were also reported have antibacterial activity by damaging bacterial cell membranes.

In this research, temu kunci was selected for further antibacterial activity test which is determination of MIC value due to the its antibacterial activity higher than others against *S. typhi*.

Table 2. The Screening Antibacterial of Methanol Extract Against *S, tyohi.*

Methanol Extract (1%)	Diameter of Inhibition Zone (mm ± SD)
Temu Putih	10,7 ± 1,2
Temu Ireng	12,7 ± 2,5
Temu Giring	19,0 ± 6
Temu Kunci	20,3 ± 2,5
Ampisilin	13 ± 0
Metanol	6 ± 0

Diameter of the well 6 mm; ampicillin (positive control, 0.25%); methanol (negative control)

Table 3. The MIC Value of Methanol Extract of Temu Kunci toward *S typhi*.

Concentration (1%)	Diameter of Inhibition Zone (mm ± SD)
0,25	13,7 ± 2,3
0,125	11,0 ± 1,0
0,0625	10,7 ± 1,2
0,03125	7,7 ± 0,6
0,0156	7,3 ± 0,6
0,0078	7,0 ± 0,0
Ampicilin	20,0 ± 0,0
Methanol	6,0 ± 0,0

Diameter of the well 6 mm; ampicillin (positive control, 0.25%); methanol (negative control)

3.3 Determination of MIC (Minimum Inhibitory Concentration) Value

Determination of the MIC value of the methanol extract of the temu kunci was carried out using the same method as the antibacterial activity screening test that had been carried out previously. The variation of the sample concentration in this study started from 1% as mother liquor and then reduced to the smallest concentration of 0.125; 0.0625; 0.03125; 0.0156 and 0.0078%. The MIC value of the methanol extract of temu kunci is shown as follows:

Based on Table 3, the methanol extract of temu kunci demonstrated the MIC value of S. typhi at the smallest concentration used in this study which was 0.0078% with an inhibition zone diameter of 7.0 mm. Therefore, the methanol extract of the temu kunci rhizome was indicated to still have the potential to inhibit the growth of the test bacteria at a lower concentration. For further studies, those methanol extracts would be investigated their effectiveness against other pathogenic bacteria strain as well as resistant bacteria.

4 Conclusion

The methanol extracts of the rhizome of temu putih, temu ireng, temu giring and temu kunci at of 1% against *S. typhi* exhibited antibacterial activity with inhibition zones of 10.7; 12.7; 19.0 and 20.3 mm, respectively. Then the MIC value of the temu kunci methanol extract against *S. typhi* was 0.0078% with an inhibition zone diameter of 7.00 mm.

Acknowledgement. The author would like to thank the head of the Laboratory of Organic Chemistry and the Laboratory of Biochemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University for the facilities during completing this research.

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