

# Antibacterial Potential of Parem Karo to Treat *Staphylococcus aureus* Infection in Diabetic Foot Patients

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## Abstract

One of the chronic and serious complications of diabetes mellitus is diabetic foot. *Staphylococcus aureus* bacteria is the most common bacteria found in this type of wound. The purpose of this study is to determine the antibacterial activity of parem Karo, which is one of the traditional medicines of the ethnic culture of the Karo tribe which is rich in spices. The results showed inhibitory activity at higher concentrations with the largest average inhibition diameter at 75% concentration against *Staphylococcus aureus* bacteria ( $8.23 \pm 0.72$  mm) followed by 50% concentration with an average inhibition diameter ( $7.52 \pm 0.2$  mm) and at 25% concentration showed no inhibition zone at all. The conclusion obtained from this study is that there is antibacterial activity of Karo hot parem extract at a concentration of 75% and 50% against the growth of *Staphylococcus aureus* bacteria.

**Keywords:** Antibacterial, Diabetes Mellitus, Diabetic Foot, Parem, *Staphylococcus aureus*.

## 1. Introduction

Diabetes mellitus (DM) is a global disease consisting of various metabolic disorders, characterized by chronically increased blood sugar levels (hyperglycemia) due to impairments in insulin secretion, insulin function, or both. Diabetic foot wounds are one of the complications that arise from high blood sugar levels in diabetes patients (Firhat et al., 2022). Microorganisms that often cause infections in this condition include *Staphylococcus aureus*, *Streptococcus*, and *Pseudomonas aeruginosa* (Roza et al., 2023). To address this condition, many diabetes patients seek alternative treatments through traditional medicine using herbal remedies, which are considered safer and have minimal side effects when used correctly (Kasole et al., 2019; Nasution et al., 2022). In Indonesia, each ethnic group has unique healing and healthcare methods based on their cultural knowledge and beliefs (Abdullah et al., 2023; Febriyanti et al., 2024; Laksono et al., 2020). One example is the use of traditional medicine from the Karo tribe, which has been passed down through generations as part of their cultural heritage to treat various diseases (Mareny, 2020).

The traditional Karo medicine referred to here is parem. Parem is a traditional medicine of the Karo tribe used to cure diseases and maintain health, which is essentially an herbal concoction made from natural ingredients with various types and properties that vary greatly (Mareny, 2020). Karo parem is divided into 2 types: cold parem and hot parem, with compositions that vary according to community needs. This study uses Karo hot parem as test material with a composition explained directly by Karo parem traders in the traditional Pancur



Batu market. This research not only has the potential to provide new treatment alternatives for diabetic foot patients but also opens opportunities for broader application of traditional medicine in addressing modern health problems. The objective of this research is to assess the antibacterial effectiveness of Karo parem, a traditional herbal medicine with spices from the Karo ethnic culture, against the bacteria responsible for diabetic foot infections.

## 2. Methods

### 2.1. Tools and Materials

The tools used in this research were analytical balance, stirring rod, incubator, Biological Safety Cabinet (BSC), rotary evaporator, autoclave, oven, water bath, magnetic stirrer, hotplate, spirit lamp, calipers, micropipette, micropipette tips, inoculating loop, evaporating dish, petri dish, tissue, cloth, writing materials, labels, test tubes, and glassware commonly used in laboratories.

The materials used in this research were Karo hot parem purchased from the traditional Pancur Batu market, Deli Serdang, North Sumatra, sterile distilled water, 96% ethanol, 10% Dimethyl sulfoxide (DMSO), nutrient agar (NA), Ciprofloxacin Antimicrobial Susceptibility Disks (5µg), McFarland 0.5, 0.9% NaCl, aluminum foil, tissue, paper discs, plastic wrapping, sterile cotton swabs, and *Staphylococcus aureus* bacterial culture.

### 2.2. Preparation of Karo Hot Parem Extract

The process of making Karo hot parem extract from the traditional Pancur Batu market, Deli Serdang, North Sumatra was carried out using the maceration method. A total of 114.19 grams of Karo hot parem powder was placed in an extraction container in the form of a glass bottle. The sample was then macerated for 5×24 hours with 96% ethanol solvent. The maceration process was carried out for 5×24 hours because the longer the extraction time, the longer the contact between sample and solvent, so the amount of extracted compounds becomes greater (Kristiana et al., 2023). Subsequently, the maceration result was filtered and evaporated using a rotary evaporator at 60°C until a thick extract was formed. The thick extract was then concentrated over a water bath until all ethanol solvent evaporated, producing a concentrated extract.

### 2.3. Antibacterial Activity Test

Antibacterial activity testing in this study was conducted using the paper disc diffusion method. Initially, sterile paper discs were saturated in test solutions of 25%, 50%, and 75%, then lifted and allowed to dry. Other paper discs were prepared for negative control using 10% DMSO, and positive control using ciprofloxacin discs (5µg). The paper discs were then placed on NA media that had been inoculated with *Staphylococcus aureus* bacterial suspension, followed by incubation at 37°C for 24 hours and then the inhibition zone was observed.



Figure 1. Karo Hot Parem Extract

### 3. Results and Discussion

Determination of antibacterial activity inhibition zone strength was based on criteria proposed by (Alouw et al., 2022), namely inhibition zones with diameters less than 5 mm are categorized as weak, 5-10 mm as moderate, 10-20 mm as strong, and more than 20 mm as very strong. The following presents the results of inhibition zone diameter measurements that have been identified.

Table 1. Results of Average Inhibition Zone Diameter Measurements of Karo Hot Parem Extract

Treatment	Inhibition Zone (mm)			Mean ± SD	Category
	Replicate 1	Replicate 2	Replicate 3		
K(-)	0	0	0	0	None
K(+)	12.47	13.19	13.91	13.14±0.72	Strong
P1	0	0	0	0	None
P2	7.41	7.41	7.76	7.52±0.2	Moderate
P3	7.70	7.93	9.06	8.23±0.72	Moderate

Description:

K(-) : 10% DMSO

K(+): Ciprofloxacin Antimicrobial Susceptibility Disks (5µg)

P1 : 25% parem concentration

P2 : 50% parem concentration

P3 : 75% parem concentration

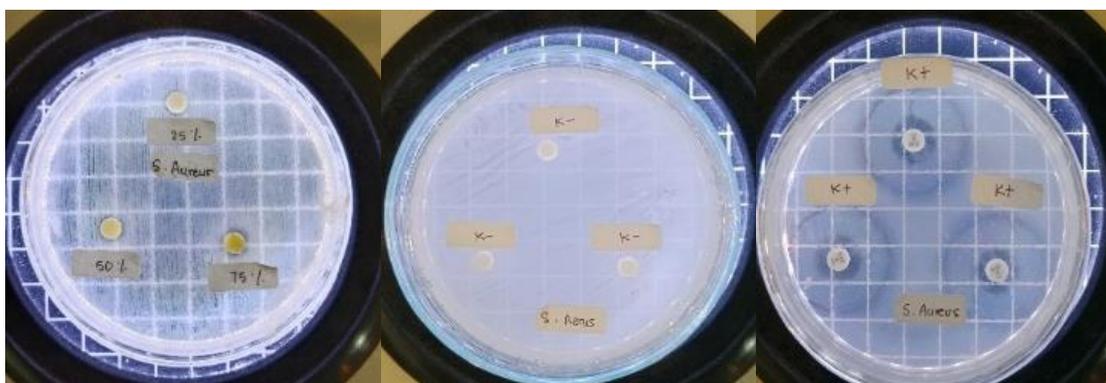


Figure 2. Inhibition Zone Diameter of Karo Hot Parem Against *Staphylococcus aureus* Bacteria

The antibacterial activity test results against *Staphylococcus aureus* showed that the negative control (10% DMSO) had no inhibitory effect, while the positive control using ciprofloxacin discs (5µg) produced an inhibition zone diameter of 13.14 mm, which falls into the strong category. At concentrations of 75% and 50%, the average inhibition zone diameters formed were 8.23 mm and 7.76 mm respectively, both falling into the moderate category. Meanwhile, at 25% concentration, no inhibition zone was formed.

The Karo hot parem used in this study is a traditional medicine of the Karo tribe purchased directly from the traditional Pancur Batu market, North Sumatra. Based on interviews with the Karo parem maker, this preparation is made from ingredients such as black pepper (*Piper nigrum*), ginger (*Zingiberis Officinalis Rhizoma*), bangle (*Zingiber purpureum*), lempuyang (*Zingiber cassumunar*), kencur (*Kaempferia galanga*), garlic (*Allium sativum*), shallots (*Allium cepa L.*), temulawak (*Curcuma xanthoriza*), temu kunci (*Boesenbergia pandurata*), and rice flour (*Oryza sativa*) ground with a stone mill. After these ingredients are mixed, the dough is formed into round shapes the size of coins, then naturally dried with sunlight for a week until completely dry. The purchased Karo hot parem was then ground using a mortar and pestle, weighed, and subsequently extracted using the maceration method.

Maceration is an extraction method that involves soaking samples or simplicia with organic solvents at room temperature (Charisma et al., 2022). The extraction process in maceration works through the breakdown of cell walls and cell membranes that occurs due to pressure differences between the inside and outside of cells. This allows secondary metabolites contained in the cell cytoplasm to dissolve in organic solvents. Proper solvent selection in the maceration process can increase extraction effectiveness, considering the solubility or polarity of active compounds in natural materials. Generally, solvents like methanol and ethanol are often used in maceration because they have a wide polarity range (Handoyo, 2020).

Testing the effectiveness of Karo hot parem extract against *Staphylococcus aureus* bacterial growth has never been conducted before. Research conducted by Sembiring et al. (2023) supports this, where they performed phytochemical screening of bioactive components in Karo Parem and obtained positive test results showing the presence of secondary metabolites such as alkaloids, flavonoids, phenols, tannins, monoterpenes, sesquiterpenes, triterpenoids, and saponins.

Several of these compounds are antibacterial agents contained in Karo parem (Sembiring et al., 2023). Antibacterial agents are substances capable of inhibiting or killing bacteria that cause infections. Infections are caused by bacteria or microorganisms that enter body tissues and multiply within them. The bacteria used in this study is *Staphylococcus aureus*, which is normal flora on the skin. When open wounds occur, this bacteria can easily enter and cause serious infections if not properly treated (Millah, 2021).

The effectiveness test was conducted to determine the extent to which Karo hot parem extract can inhibit *Staphylococcus aureus* growth. Research results showed that at 75% concentration, the average inhibition zone diameter was 8.23 mm, at 50% concentration the average inhibition zone was 7.52 mm, while at 25% concentration there was no inhibition zone at all. These results indicate that the higher the extract concentration, the larger the inhibition zone formed. The increase in inhibition zone diameter in this study is proportional to the increasing extract concentration given during testing. Research conducted by Hasanah et al. (2023) stated that antibacterial effectiveness in inhibiting microorganisms depends on the concentration level and type of antibacterial agent.

Optimal results in the antibacterial effectiveness test against *Staphylococcus aureus* by Karo hot parem extract were found at 50% and 75% concentrations, with average inhibition

zone diameter values of 7.52 mm and 8.23 mm respectively. Ciprofloxacin discs (5µg) used as positive control produced an average inhibition zone diameter value of 13.14 mm, which is classified as strong. Although Karo hot parem extract has not been able to exceed the antibacterial activity of ciprofloxacin as positive control, these results indicate that the extract can serve as an alternative treatment.

#### 4. Conclusion

Based on the research results, Karo hot parem extract shows antibacterial activity against *Staphylococcus aureus* growth, indicated by the formation of clear zones around the paper discs. The largest inhibition zone was found at 75% concentration with a diameter of 8.23 mm and at 50% concentration of 7.52 mm, both classified in the moderate category. However, at 25% concentration, no inhibition zone was formed at all. Although the extract shows antibacterial activity, its inhibitory power is still not comparable to ciprofloxacin disc (5µg) as positive control.

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