



Antibacterial Effectiveness of Golden Garlic Against Gram-Positive Bacteria from Diabetic Ulcer Patients with High CRP Levels

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Abstract

The wrong antibiotic therapy in patients with DM results in therapeutic failure and increases the risk of antibiotic resistance. Herbal ingredients can be used as antibacterial alternatives to prevent the growth of Gram-positive bacteria so that there is no resistance and further complications from diabetics. One of the natural antibacterial alternative herbs is Golden garlic or golden coloured garlic extract. Golden garlic contains the bioactive component Allisin as an antibacterial on several pathogenic bacteria, such as Gram-positive bacteria. Based on this, the purpose of the study was to analyze the Antibacterial Effectiveness of Golden Garlic Against Gram-Positive Bacteria From Patients With Diabetic Ulcers With High CRP Levels. Research design with analytical observational method and cross sectional research design, purposive sampling technique. Samples with high CRP from diabetic ulcer swabs were 10 samples with the results of *Staphylococcus aureus* bacterial growth and tested the effectiveness of golden garlic ethanol extract with concentrations of 100%, 80%, 60% and 40%. The results of the study of golden garlic extract with Cindamycin positive control showed intermediate effectiveness in inhibiting the growth of Gram-positive bacteria with a concentration of 100%, while with concentrations of 80%, 60% and 40% showed more resistance. Analysis with SPSS Anova there is a difference in effectiveness by showing the inhibition zone of Golden Garlic with a concentration of 100%, 80%, 60% and 40% against Gram Positive Bacteria from Diabetic Ulcer Patients with High CRP Levels of 0.000. The results showed that Golden Garlic extract can be an alternative to antibacterial herbs in diabetic ulcers.

Introduction

Degenerative diseases are the inability of the body's organs to function properly, usually occurring during old age but can also occur at a young age, the result is a decline in health which is generally followed by the appearance of disease. The threat of these diseases is expensive pain and can lead to death. Degenerative diseases do not occur suddenly, but develop slowly over time. They are also influenced by unhealthy lifestyles and diets. Degenerative diseases, one of which is type 2 diabetes mellitus, is caused by insulin deficiency (Lima et al., 2022; Kaur et al., 2018). Insulin deficiency causes the body to be unable to utilise glucose properly. This causes blood sugar levels to rise, which is called hyperglycaemia (Aliviameita et al., 2021; Alamri et al., 2019; Balaji et al., 2019).

Diabetes mellitus (DM) is a chronic disease characterised by the body's inability to metabolise carbohydrates, fats and proteins, resulting in elevated blood sugar levels (hyperglycaemia)

(Mukhtar et al., 2020; Tripathi & Srivastava, 2006; Jangid et al., 2017). The number of people suffering from diabetes mellitus is increasing due to changing lifestyles. The number of people suffering from diabetes in Indonesia increased from 8.4 million in 2000 to approximately 21.3 million in 2030. The morbidity rate in Indonesia is so high that the country is ranked 4th in the world (Susanto et al., 2009; Titaley et al., 2008).

A common complication that often occurs in people with diabetes is foot problems. It is the main cause of high morbidity and mortality in people with diabetes (Wang et al., 2022; Stancu et al., 2022). The prevalence of people suffering from diabetic foot wounds in Indonesia is around 15%, the number of amputations is 30%, the mortality rate is 32% and diabetic foot wounds are the main reason for hospitalisation at 80% for people with diabetes. This is supported by the fact that the number of people suffering from diabetic foot wounds in Indonesia is increasing, with an 11% increase in prevalence (Novianti et al., 2020).

Diabetic ulcer is one of the most dreaded chronic complications of diabetes mellitus. Most patients with diabetes mellitus will develop diabetic ulcers, with a percentage between 2-10% each year. And about 15-25% of those diabetic patients will develop diabetic ulcers during their lifetime. Diabetic ulcers can become infected as germs or bacteria enter and high blood sugar allows the growth of germs. Severe infection of the soft tissue and bone often results in amputation. After undergoing amputation, the patient's condition does not always improve. About 14.3% of patients will die within one year after amputation, and about 37% of patients will die within three years after amputation. Half of the amputations are caused by infections in the wound that cannot be treated. One of the reasons for many amputations is the misuse of antibiotics (Novianti et al., 2020; Yang et al., 2022).

Management of diabetic wound infections requires the use of antibiotics as one of the effective treatment methods. Antibiotics are most commonly used to treat bacterial infections. Inappropriate antibiotic administration can cause several problems, such as increased microbial resistance to single or combined antibiotics, increased toxicity due to incorrect antibiotic selection and dosage, and side effects that make infection healing longer, especially in patients with diabetes mellitus (Antari & Esmond, 2017).

Healing of diabetic ulcers is very important, therefore there is a need for natural herbal ingredients as antibacterials that can replace antibiotics so that patients with diabetic ulcers do not depend on antibiotics that can cause resistance. Research has been conducted on the ability of garlic as an antibacterial against several types of bacteria from herbal plants. However, there is no information on whether garlic is also effective as an antibacterial against bacteria that cause diabetic gangrene. Thus, it is important to study how Golden garlic acts against bacteria that cause diabetic gangrene in patients with high CRP levels (Antari & Esmond, 2017).

Patients with diabetes mellitus with high CRP levels can be used as a marker of active acute inflammation (Salima, 2015), this occurs due to the activity of Gram-positive bacteria in the ulcer. CRP is an inflammatory marker, this material is a protein formed in the liver that functions as a non-specific monitor. Examination of CRP levels can be used to see inflammatory activity, CRP levels in inflammatory conditions will increase 100x or more than 100x if it occurs after trauma, microorganism infection including bacteria. The results of high CRP levels as a marker of persistent infection and inflammation (Salima, 2015).

Garlic contains bioactive compounds that act as antibacterial on some pathogenic bacteria. The use of antibiotics is one way to treat infections. However, if their use is not done wisely, this can cause new problems, namely the emergence of antibiotic-resistant bacteria. Based on the information above, there are thoughts that encourage research on the test of antibacterial effectiveness of Golden Garlic against Gram-positive bacteria in patients with diabetic ulcers with high CRP levels. Research in the field of immunoserology and microbiology. In the field

of infectious diseases of microorganisms that cause ulcers in degenerative diseases (diabetes mellitus) is one of the research focuses at IIK Bhakti Wiyata.

Methods

In this study, the method used was observational analytic with cross sectional design. The study was conducted to collect information about patients with type 2 diabetes mellitus who have diabetic ulcers in the Kediri area. The population and samples used in the study were patients with diabetic ulcers in Kediri City. The sampling technique used in this study was purposive sampling. The criteria for including in the sample are people who suffer from diabetes for more than 5 years and are not having a fever. The data used in this study are data on patients with type 2 diabetes mellitus who have ulcers in Kediri City.

Samples were taken from ulcers with diabetes mellitus with screening test of high CRP levels. CRP examination was carried out with the latex agglutination method conducted at the immunoserology laboratory IIK Bhakti Wiyata Kediri with serum samples. CRP examination was carried out with qualitative and semi-quantitative methods with serum samples taken with CRP levels of more than 6 IU/ml. Effectiveness Test by making Golden garlic extract as much as 1000 grams Golden garlic is aerated to dry, not exposed to sunlight and then ground until it becomes a powder weighing 200 grams, then soaked in 96% ethanol for one week, stirring every day. The soak was put into the extraction tool, the extraction results will be obtained thick liquid, brown in colour, smells aromatic. Extraction results were diluted with concentrations of 100%, 80%, 60% and 40% with DMSO.

Swab results of diabetic ulcers with high CRP results are then carried out identification of Gram-positive bacteria with BAP media, MSA and Gram staining. After obtaining the results of Gram-positive bacteria, the effectiveness of golden garlic disc diffusion method was tested with concentrations of 100%, 80%, 60% and 40%. The data obtained was then analysed using SPSS one way Anova.

Result and Discussion

This study took samples from patients with diabetic ulcers with high CRP levels, from the high CRP examination there were 10 samples swabbed in diabetic ulcers. From the swab 10 samples continued with culture examination with BAP and MSA media and Gram staining. The laboratory results are as follows:

Table 1. Identification of Gram-positive Bacteria with High CRP Levels

Sample Code	CRP Result	Identification of Gram-positive Bacteria
A	24	+
B	24	+
C	12	+
D	24	+
E	12	+
F	48	+
G	24	+
H	12	+
I	48	+
J	24	+

Description: + = Gram positive bacteria: Staphylococcus aureus bacteria



Figure 1. Gram Positive Bacteria on BAP Media, MSA Media and Gram Stain

Table 2. Effectiveness of Golden Garlic with Concentrations of 100%, 80%, 60% and 40%

Sample	CRP	Golden Garlic				Positive Control Clindamycin Antibiotic
		100	80	60	40	
A	+	I	R	R	R	S
B	+	I	R	R	R	S
C	+	I	I	R	R	S
D	+	I	I	R	R	S
E	+	R	R	R	R	I
F	+	I	R	R	R	S
G	+	I	I	R	R	S
H	+	I	R	R	R	S
I	+	I	R	R	R	I
J	+	I	I	R	R	I

Description:

I = Intermediates

R = Resistance

S = Sensitised

Table 3. Diameter of Golden garlic effectiveness test with 100%, 80%, 60% and 40% concentration

Sample	CRP	Golden Garlic				Positive Control
		100	80	60	40	
A	24	16,4	9,6	12	9,4	21,6
B	24	17,6	14,6	12,4	9,6	28,2
C	12	16,3	15	12,4	9,3	22
D	24	16,3	14	10,5	9,5	22
E	12	14	12,5	9,7	8,5	20,5
F	48	16,4	14,6	12	9,3	22,7
G	24	17	15	14	11	22,4
H	12	16,2	14	11,6	9	21
I	48	17	12	10,3	9	20
J	24	16	15	12	8	20

Sensitive: ≥ 21 mm

Intermediate: 15-20 mm

Resistant: ≤ 14 mm

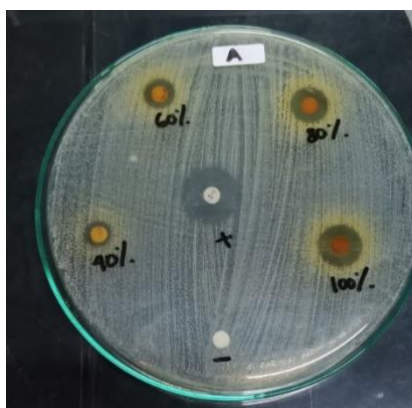


Figure 2. Golden Garlic Effectiveness Test with Concentrations of 100%, 80%, 60% and 40%

Based on statistical tests with one way ANOVA, the results obtained with a value of ($\alpha < 0.05$) that there are differences in the effectiveness of each concentration in the Golden Garlic antibacterial effectiveness test against Gram-positive bacteria from diabetic ulcer patients with high CRP levels. The 100% concentration of golden garlic extract showed the results of 9 intermediate samples and 1 resistant sample, 80% concentration of golden garlic extract 3 intermediate samples and 7 resistant samples, while 60% and 40% concentrations showed all resistant samples. Our positive control used Clindamycin antibiotic which according to CLSI 2024 showed 7 sensitive samples and 3 intermediate samples.

The study was conducted to analyse the test of antibacterial effectiveness of golden garlic against Gram-positive bacteria from patients with diabetic ulcers with high CRP levels. Golden garlic extract contains active compounds that can inhibit the growth of Gram-positive bacteria so that an inhibition zone will form around the disc. The area that is inhibited is then measured at each concentration. In a study conducted by Fahmi, Andriana, and Hidayati, it was mentioned that the size of the diameter of the inhibition zone formed by traditional medicines is considered sensitive if the diameter of the inhibition zone is 18 mm, intermediate if the diameter of the inhibition zone is 13-17 mm, and resistant if the diameter of the inhibition zone is 12 mm. Based on that statement, extra golden garlic with a concentration of 100% in this study is classified as intermediate and is still able to inhibit the growth of Gram-positive bacteria with an inhibition zone diameter of 14-17.6 mm formed around the colony of Gram-positive bacteria (Dewi et al., 2022; **Mardiyah, 2018**). The 80% concentration still has 3 samples that are still able to inhibit Gram-positive bacteria while the 60% and 40% concentrations of the inhibition zone have a diameter of 8-12.4 mm and are categorised as resistant. The presence of inhibition zones is due to the chemical compounds contained in golden garlic extract, namely essential oils, saponins, flavonoids, ajoene, alicin which act as antibacterials.

According to Dewi, essential oils, tannins, and flavonoids affect bacteria by causing changes in protein molecular stability and protein structure. This can damage the cell's cytoplasmic membrane, cause damage to the cell wall, and inhibit bacterial growth. Denatured proteins lose their secondary and tertiary structures due to pressure and heat from compounds such as alcohol and chloroform, which can destabilise the bacterial cell wall and cell membrane. This can interfere with the ability of the cell wall to be selective in permeability, active transport function, and control of the protein arrangement of bacterial cells. The integrity of the cytoplasm is damaged, large substances and ions escape from the cell. Bacterial cells undergo lysis as they lose their shape (Sulfianti et al., 2023; **Fahmi et al., 2019**; **Sulistiyowati, 2015**).

Golden garlic extract with a concentration of 40%-100% has no inhibitory effect on this sensitive diameter because factors such as diffusion rate, number of microorganisms, bacterial growth rate, and conditions during incubation affect the diameter of the inhibition zone produced. In addition, there is another factor, namely the administration of antibiotics before sampling, which can affect the number of bacteria inoculated. The results of this study can be used as a guideline to use different solvents, different extract concentrations, examine other parts of the garlic plant, and conduct tests on bacteria with different genus or species. It is recommended to use chromatography when isolating the antibacterial substances from garlic that most effectively inhibit bacterial growth (Bar et al., 2022; Torres et al., 2021).

Conclusion

There are differences in the Antibacterial Effectiveness of Golden Garlic Against Gram-Positive Bacteria From Diabetic Ulcer Patients With High CRP Levels with a value of ($\alpha < 0.05$), namely there are differences in the inhibition zone of golden garlic extract with the highest order of optimal concentration of 100% followed by concentrations of 80%, 60% and the lowest 40%. Golden garlic extract can be used as an antibacterial alternative for the treatment of diabetic ulcers.

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