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THE EFFECTIVENESS OF *CLITORIA TERNATEA* FLOWER EXTRACT TO INHIBIT THE GROWTH OF *SALMONELLA TYPHI* BACTERIA THAT CAUSES TYPHOID FEVER INFECTION

***Nadya Treesna Wulansari and Luh Yenny Armayanti**

Institute of Health Science Bali, Jl. Tukad Balian No 180, Denpasar, Bali, Indonesia

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ABSTRACT

Typhoid fever is one of the endemic diseases that show a tendency to increase every year. This disease is caused by *Salmonella enterica serotype typhi* and *paratyphi*. However, the resistance of *Salmonella typhi* against antibiotic usage can lead to antimicrobacterial resistance or multiple drug resistance. Resistance of some strains of *Salmonella typhi* by using antibiotics in the long term can be harmful for the patient. This study aims to determine the effectiveness of *Clitoria ternatea* flower extract to inhibit the growth of *Salmonella typhi*. Research was conducted at Microbiology Laboratory, Technology Agriculture Department Udayana University, Denpasar Bali-Indonesia. The concentration of flower extract at concentration 20%, 40% and 60% in inhibit the growth of *Salmonella typhi* were 2.97 ± 0.39, 4.90 ± 0.40 and 7.78 ± 0.5 mm. The higher extract of concentration can give stronger antibacterial characteristics in inhibiting bacterial growth. Therefore, *Clitoria ternatea* flowers can be used as an alternative traditional medicine to inhibit the growth of *Salmonella typhi* which cause typhoid fever. In addition, typhoid fever can be prevented by several steps such as maintaining the cleanliness of food and beverage production, environmental sanitation, health education and vaccination.

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INTRODUCTION

Typhoid fever or typhoid abdominatis is a disease of systemic broad spectrum clinical infection caused by *Salmonella enterica serotype typhi* and *paratyphi* (Andino and Hanning, 2015). The disease is capable of infecting more than 17.000.000 people annually and makes over 600.000 deaths worldwide (WHO, 2009). Typhoid fever infections are very rapid, especially in developing countries like Indonesia which has a tropical climate (Lauria, 2009). According to data from *Riset Kesehatan Dasar* (2007), typhoid fever caused 1.6% of the deaths of Indonesians of all age groups. In Indonesia typhoid fever is one of the endemic diseases that shows a tendency to increase every year with an average infection of 500 / 100.000 population with the percentage of death of 0.6-5% (*Menteri Kesehatan Republik Indonesia*, 2006). Typhoid fever is one of the major health problems in endemic areas that can occur due to lack of sanitation from food and beverage management. The problem is one of the factors causing the emergence of cases of typhoid fever in Bali (Verry, 2014). Besides that, typhoid fever may be caused with close contact with typhoid cases or carries, level of education, travelling to endemic areas, rainfall, vapour pressure and temperature (Tran, 2005; Whitaker, 2009 ; Kelly, 2007 ; Wang, 2012). During this time, the prevention of typhoid fever is by giving antibiotics. The use of broad spectrum antibiotics over the past two decades has led to new problems of antibiotic resistance, especially in non procedural and uncontrolled treatment (Kelanit *et al*., 2016). Incorrect antibiotics may cause microorganisms to be immune to one (antimicrobacterial resistance) or some type of antibiotic (multiple drug resistance) (Utami, 2012). This can lead resistance to some strains of *Salmonella typhi* bacteria (WHO, 2003). Resistance to pathogenic bacteria by using antibiotics in the long term can be detrimental to the patient. Efforts to prevent the negative impact of synthetic drugs based on chemicals of infectious diseases can be done with the use of plants or natural materials as a drug known as traditional medicine. Traditional medicines

Corresponding author:* **Nadya Treesna Wulansari,
Institute of Health Science Bali, Jl. Tukad Balian No 180, Denpasar, Bali, Indonesia.

have been used long time ago by the people of Indonesia because it is very beneficial to health and it does not cause side effects for the body. Plants are used as traditional medicines having secondary metabolites such as terpenoids, tannins, alkaloids, flavonoids, saponins and antraquinones have been found to have antimicrobial activity in vitro (Darsini and Shamshad, 2013). One of the plants that can be used as a traditional medicine is *Clitoria ternatea* flower. *Clitoria ternatea* plant shows the effects of pharmacology such as antioxidant, anticancer, anti inflammatory, antipyretic, antidiabetic, antimicrobial, antiparasitic, gastrointestinal, analgesic, insecticidal and others (Snafi, 2016). Phytochemical *Clitoria ternatea* flowers are also found to contain flavonoids, alkaloids, and terpenoids (Darsini and Shamshad, 2013). In addition Uma *et al* (2009) states that, crude methanol extract of blue *Clitoria ternatea* flowers can inhibit the growth of pathogenic bacteria that infect the urinary tract. Given the magnitude of the potential of these plants and the benefits it contains, it is highly probable that the flower extract of *Clitoria ternatea* has the potential as an antibacterial in inhibiting the growth of *Salmonella typhi* causes typhoid fever.

MATERIALS AND METHODS

Plant material and *Salmonella typhi* Bacteria

Clitoria ternatea flower was collected from Denpasar, Bali-Indonesia. Bacteria was obtained from Microbiology Laboratory, Technology Agriculture Department Udayana University, Denpasar Bali-Indonesia.

Extraction

Clitoria ternatea flower was extracted successively with aquadest solvent and made variation of extract concentration 20%, 40%, and 60%.

Activity Assay

Antibacterial activity assay was conducted using diffusion well method (Balouri *et al*., 2016). Nutrient agar plate surface is inoculated by spreading a 100 µL volume of the *Salmonella typhi* inoculums over the entire agar surface. Then, a hole with a diameter of 5 mm is punched aseptically with a sterile cork borer and volume 20 µL of the extract *Clitoria ternatea* flower at desired concentration is introduced into the well. Then, agar plates are incubated in 37^0C for 24 hours. The diameter of inhibition zone (mm) will be observed through callipers.

Phytochemical test

Qualitative and quantitative screening of secondary metabolite compounds was performed to examine the presence of phenol, flavonoid and tannin compounds present in the flower extract of *Clitoria ternatea* (Chakraborty *et al*., 2017).

Statistical analysis

The main data on antibacterial activity test using the diffusion well method obtained in this study will be analyzed by variance analysis (ANOVA) (Pallant, 2010).

RESULTS

The effectiveness of *Clitoria ternatea* flower extract to inhibit the growth of *Salmonella typhi* bacteria was measured using the diameter of the inhibition zone produced by the extract. The increase of concentration of extract used in this study started from concentration 20%, 40%, and 60%. The diameter of the resistance zone generated from this flower extract is the main data in this study. Positive control in this research used ciprofloxacin and negative control used aquadest solvent. The zone of obstruction or an empty area around the diffusion well is caused by the content of bioactive compounds contained in the *Clitoria ternatea* flower. The average diameter of the flower barrier extract resistance zone to the growth of bacterial isolates of *Salmonella typhi* is shown by Table 1 below.

Table 1. Mean of Barrier Zone Diameter of *Clitoria ternatea* flower extract on Growth of *Salmonella typhi*

Extract Concentration	Mean of Barrier Zone Diameter (mm)**
20%	2.97 ± 0.39b
40%	4.90 ± 0.40c
60%	7.78 ± 0.50d
Positive control	20.46 ± 0.43e
Negative control	0.00 ± 0.00a

Information
** The values in the standard deviation ± table are the average of six repetitions. Different alphabet in the Table show significantly different results (p <0.05), based on Duncan test after ANOVA analysis

The data in Table 1 showed that the flower extract has varying degrees of resistance. In general, mean inhibition power of flower extract was significantly different (p <0.05) with positive control and negative control. In addition, the mean inhibitory power of all variations of flower extract concentration in the *Salmonella typhi* bacteria was significantly different (p <0.05) from each other (Table 1). Concentration of flower extract of *Clitoria ternatea* at concentration 60% most effective in inhibiting growth of *Salmonella typhi* bacteria that is equal to 7.78 ± 0.50 mm.

Table 2. Phytochemicals Screening Flower Extracts of *Clitoria ternatea*

Phytochemicals	Result	Value
Fenol	Presence	1.70 mg/100 ml GAE
Flavonoid	Presence	8.76 mg/100 ml QE
Tanin	Presence	3.02 mg/100 ml TAE

The data in Table 2 show that the flower extract of *Clitoria ternatea* contains secondary metabolite compounds of phenol, flavonoids and tannins. Highest levels of secondary metabolite compounds are flavonoids is equal to 8.76 mg / 100 ml QE.

DISCUSSION

Inhibition of *Salmonella typhi* bacteria growth with increased concentration of flower extract of *Clitoria ternatea* proved the difference in research that has been done. This difference was obtained by using parametric test of Analysis of Variance One Way and Duncan test with significance number <0.05 is 0.000. This indicates that there is a significantly different effectiveness effect on increasing the concentration of flower extract on the growth barrier of *Salmonella typhi* bacteria. The concentration of extract greatly affect the work of an antibacterial, the higher the concentration of extract given the stronger the antibacterial properties in inhibiting bacterial growth (Keyser *et al*., 2005). *Salmonella typhi* is a type of bacteria belonging to the Enterobacteriaceae family, rod shaped, belonging to gram negative bacteria that are not

encapsulated and having a length of 2-3 µm and a diameter of 0.4-0.6 µm (Jawetz, 2008). These bacteria have pathogens that cause conditions of *Salmonella* bacteremia which are able to penetrate the intestinal epithelial mucosa and enter to the bloodstream (Shu-Kee Eng, 2015). Gastroenteritis is a manifestation of *Salmonella* infection followed by bacteremia and enteric fever (Majowicz *et al*., 2010). Flower of *Clitoria ternatea* is one of the plants which function as traditional medicine. For people in Penglipuran Village, Bangli Regency, this extract is consumed and used as a traditional drink known as "*loloh telang*". The effectiveness of *Clitoria ternatea* flower extract inhibiting *Salmonella typhi* growth is caused by the bioactive compound contained in the flower of the aperture. Phytochemical of *Clitoria ternatea* flowers also found to contain flavonoids, alkaloids, terpenoids, saponins, proteins, steroid, anthtroquinones (Darsini and Shamshad, 2013; Valivittan and Isaac, 2016). In addition Uma *et al.* (2009) states that, crude methanol extract of blue *Clitoria ternatea* flowers can inhibit the growth of pathogenic bacteria that infect the urinary tract. Anthocyanins contained in petal flower petals have antioxidant activity and are able to inhibit the growth of *Penicillium citrinum* (Kungsuwan *et al*., 2014; Priprem *et al*., 2014).

Based on the qualitative screening of the metabolite compound the extract contains phenols, flavonoids and tannins. Phenol is a compound capable of inactivating proteins in cell membranes, destroying and penetrating cell walls inhibiting bacterial growth (Novita, 2016). Flavonoids are able to interact with bacterial genetic material that causes damage to the permeability of cell wall bacteria, microsome and lysosomes and inhibit the metabolism of energy and bacterial motility (Cushnie, 2005; Darsana *et al*., 2012). In addition, the mechanism of action of flavonoids in inhibiting the function of cell membranes by forming complex compounds of extracellular and dissolved proteins there by damaging the bacterial cell membranes that cause the release of intracellular compounds in bacterial cells (Li, 2003). The tannin compound has a mechanism of coagulating and denaturing proteins (Yulia, 2006). Tannins bind to proteins to form H^+ ions, causing the pH to become acidic so that the protein is denatured. The acidic conditions inactivate enzymes in bacteria and cause disturbed metabolism and cellular damage and even death. Tannins can inhibit the enzyme reverse transcriptase and DNA topoisomerase so that bacterial cells can't form (Robinson, 1995).

The use of *Clitoria ternatea* flower can be used as an alternative traditional medicine in inhibiting the growth of *Salmonella typhi* bacteria that causes typhoid fever. In addition, typhoid fever can be prevented by several steps such as maintaining the cleanliness of food and beverage procurement, environmental sanitation, health education and vaccinations (WHO, 2003). The largest transmission route of typhoid fever is consuming foods and drinks that have been contaminated with *Salmonella typhi*. Food sources that can be transmitted to Salmonella are fresh fruits and vegetables (Pui *et al*., 2011). Handling and processing of food and beverages can be done by doing processes such as washing hands with soap before contact with the food and also the food must be cooked. Therefore, maintaining the safety and cleanliness of drinking water is the main way to eliminate the transmission route *Salmonella typhi* (Shu-Kee Eng, 2015). In addition proper sanitation greatly contributes to reduce the risk of transmission of all diarrheal pathogens including *Salmonella typhi*. Provision of health education for the community is very important in order to raise awareness and change the behavior of the community in taking precautions to infect typhoid fever.

Conclusion

Flower extract of *Clitoria ternatea* can be used as an alternative traditional medicine in inhibiting the growth of *Salmonella typhi* bacteria that cause typhoid fever. In addition, typhoid fever can be prevented by several steps such as maintaining the cleanliness of food and beverage procurement, environmental sanitation, health education and vaccinations.

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