Antibacterial activities of *Physalis angulata* herb extract on white feces diseases (WFD) in *Litopenaeus vannamei*

E Saraswati\(^1\*)\(^,\) AS Wijaya\(^1\)

\(^1\)Faculty of Agriculture and Fisheries, University of 17 Agustus 1945 Banyuwangi

Corresponding author Email: erikasaraswati@ymail.com

Abstract. *Litopenaeus vannamei* shrimp is one of the leading export commodities in fisheries from Indonesia, however White Feces Diseases (WFD) cause deaths up to 30% and decrease their growth and production. WFD is caused by the accumulation of several *Vibrio* bacteria in shrimp water and intestines. One of the local herbs that has the potential as antiproliferation, antidiabetic, anticytotoxic commonly used in the treatment of humans diseases is *Physalis angulata* known as ciplukan. The aim of this study was to obtain ciplukan extract which has an antibacterial activity of *Vibrio* sp., and to analyze the anti-bacterial effect of ciplukan extract on *Vibrio* bacteria from *vannamei* infected with WFD. This study used descriptive and experimental methods using a randomized block design. Data collection methods were carried out through direct observation of the object under study. Observation data were analyzed by F-test followed by HSD. The results showed that the extract of ciplukan plants contained antibacterial active ingredients. Chloroform solvent extracts had the best antibacterial activity with 13 mm inhibition zone on green bacterial colonies and 16.5 mm in yellow bacterial colonies derived from shrimp intestinal infected with WFD. The best concentration that provides the highest inhibition zone as obtained by 10% chloroform extract.

1. Introduction

White Feces Diseases (WFD) is one of the diseases that are quite troubling for shrimp farmers in Indonesia. This disease commonly called white defecation generally attacks shrimp at breeding ages of 30 - 80 days, which is marked by the release of white shrimp stools that float on the surface of the water, shrimp appetite decreases, slow growth and chronic death [1]. WFD is closely related to the group *Vibrio* spp. [2]. The types of *Vibrio* bacteria found in WFD include *V. parahaemolyticus* (green colonies), *V. fluvialis* (yellow colonies), *V. vulnificus* (green colonies), *V. mimicus* (green colonies), *V. alginolyticus* and *V. cholera* (non 01) [1]. Shrimp intestine infected by WFD is not filled with food but contains white stool strands. The impact of WFD on shrimp is a decreased appetite, growth inhibition and chronic death [2].

Efforts made by shrimp farmers to control WFD recently are by controlling the growth of *Vibrio* spp bacteria both in the water of shrimp culture and in shrimp. One alternative to many natural ingredients found around aquaculture and medical properties is *Physalis angulata* known as ciplukan.

Ciplukan is one herb that is often found in various places in the tropics, including Indonesia. Phytochemically, ciplukan plants contain saponins, flavonoids, steroids, polyphenols, alkaloids, flavonoids, and others. The content of these natural ingredients has quite good antimicrobial activity, namely as an anti-cancer [3]; anti-ulcer [4]; antimicrobial [5]; antiproliferation [6]. Based on several references it can be seen that ciplukan plants potential as a source of bioactive compounds that are useful as laxatives, boil medicines, bleeding gums, gonorrhea, diabetes, inflammation, and so on. The
The activity of *P. angulata* L. oil extract is antimicrobial and antifungal, with higher antifungal activity [7]. Goztok, F., Zengin, F (2013) found that *P. peruviana* fruit extract has antibacterial and antifungal properties which can be used as an antimicrobial agent in the development of new drugs to control infectious diseases [8]. The methanol extract from *P. angulata* has antimicrobial activity against *Bacillus subtilis* [9]. Water and ethanol extracts from *P. angulata* inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* [10]. Some of the results of these studies generally focus on diseases that attack humans, so that the effect on bacterial disease in shrimp is unknown. Therefore, it is necessary to do research to examine the potential of ciplukan plant extract against *Vibrio* spp. in shrimp infected with WFD. The purpose of this study was to obtain ciplukan extract which has an antibacterial activity of *Vibrio* sp., analyzed the anti-bacterial effect of ciplukan extract on vannamei infected with *Vibrio* bacteria of WFD.

2. Methods and materials

The materials used in this study were ciplukan plants taken from the ponds and yards in Banyuwangi, Indonesia. This study also collected ethanol, chloroform, n-hexan, ethylacetate, *Vibrio* bacteria from vannamei shrimp infected with WFD, and others.

The method used in this study is a descriptive method and experimental design with a Randomized Block Design. Descriptive method is used to find out the solvents that produce ciplukan extract which can inhibit the growth of WFD bacterial isolates. The experimental method was used to determine the antibacterial inhibition of selected extracts. The treatment applied was to figure out the concentration differences of ciplukan extract which was done repeatedly for four (4) times with groups of green bacterial colonies from the intestine, yellow bacterial colonies from the intestine, colonies of green bacterial and yellow bacterial colonies from the water of shrimp culture infected with WFD.

The collected ciplukan plants were cleaned from dirt, separated between the stems, leaves and roots of the plants, and then dried. After drying the plant sample, it was crushed with a grinder and stored in a closed place. A total of 50 grams of ciplukan flour macerated with levels of N-hexan, chloroform, ethyl acetate and ethanol each 500 mL (1:10), stored for two days. The extract is separated from the pulp by filtering it and then dried.

*Vibrio* spp bacterial isolates obtained through bacterial culture derived from water of shrimp culture and from intestinal shrimp infected with WFD. The culture results were isolated from each of the colonies of green bacteria and yellow colonies for antibacterial testing and inhibition zone test.

3. Results and discussion

3.1. Anti-bacterial activity for white feaces diseases

Antibacterial activity test was used to determine the antibacterial activity of each extract obtained by solvent N-hexan, Chloroform, Ethyl Acetate and Ethanol. In this study, antibacterial tests were carried out on ciplukan leaf and stem extracts against yellow bacteria and green bacteria isolated from shrimp intestines and shrimp culture water that infected of WFD. The results showed that each extract obtained had different anti-bacterial activity against *Vibrio* Spp bacterial colonies as shown in Figure 1. below.
Figure 1. Antibacterial test results of stem and leaves extract of Ciplukan against *Vibrio* Spp. Bacteria colonies from water of shrimp culture infected by WFD.

Based on the results of the antibacterial test it was found that the extract giving the highest antibacterial activity was ciplukan extract with chloroform solvent followed by ciplukan ethyl acetate extract. Antibacterial activity of ciplukan extract in green colonies is relatively lower than in yellow colonies. This is because the pathogenicity of *Vibrio* green bacteria is higher than that of yellow colonies.

Figure 2. Antibacterial test results of ciplukan stems and leaves extracts on green colonies and yellow colonies *Vibrio* spp. from shrimp intestine infected with WFD.
Based on the results of antibacterial tests it can be seen that the extract which provides a high enough inhibitory effect is extracts with chloroform and ethyl acetate solvents, both from the stem and leaves, to the *Vibrio* bacterial isolates green colonies and yellow colonies as the result from isolation of the intestine and water of shrimp culture infected by WFD. The inhibition zone of extracts in green colonies is lower than in yellow colonies. This shows that the resistance of green colony bacteria is higher than that of yellow colonies. *Vibrio* bacteria in green colonies are generally *Vibrio* bacteria that have the potential to cause disease in shrimp farming such as *V. harveyi*, and *V. parahaemolyticus*. While yellow colonies are generally *Vibrio* bacteria that are able to ferment sucrose to acid and reduce pH such as *V. cholera* and *V. alginolyticus*. Green colonies can be said as bacteria that are more pathogenic than yellow colonies in shrimp farming. It can be stated that the extract of ciplukan with chloroform solvent is able to inhibit the activity of *Vibrio* spp. Some argue that the bacterial inhibition zone which is less than 5mm has weak inhibitory activity, the inhibition zone between 5 - 10 mm is categorized as moderate, the inhibition zone ranging from 10 to 19 mm is categorized as strong and the inhibition zone ranging from 20 mm or more, is considered very strong.

![Figure 3](image.png)

**Figure 3.** Comparison of inhibition zone of ciplukan extract with Chloroform solvent on *Vibrio* green colonies (A) and yellow colonies (B).

### 3.2. Ciplukan extract inhibition zone test

The inhibitory test of ciplukan extract was carried out on extract of ciplukan extracted with chloroform and ethyl acetate solvents which had the highest anti-bacterial activity compared to other solvents. The inhibitory test was carried out on the bacteria *Vibrio* green colonies and yellow colonies which were isolated from water of shrimp culture and intestines of shrimp infected by WFD.

The results of variance analysis on the inhibition zone of ciplukan stem extracts with different concentrations on the growth of *Vibrio* spp bacteria showed that the difference in concentration greatly affected the inhibition zone of ciplukan extract. The greater the concentration followed by the increase of the inhibition zone of extracts up to 15% concentration and it decreased then. The best inhibition zone was obtained at concentration of 5% which was 11.5 mm and was not different from concentrations of 10, 15 and 20%. Higher concentrations are not chosen as the best concentration because the inhibition zone is still in the same category that is strong.
Table 1. Results of variety analysis of inhibition zone of ciplukan extract with chloroform solvent on Vibrio spp. bacterial isolate from vannamei intestine infected by WFD.

| Treatment Extract concentration | Average inhibition zone (mm) | Notation (p>0.01) |
|--------------------------------|------------------------------|-------------------|
| 0%                             | 6.5                          | A                 |
| 5%                             | 11.75                        | B                 |
| 10%                            | 13                           | B                 |
| 20%                            | 13.25                        | B                 |
| 15%                            | 14.25                        | B                 |

In contrast to the results of the ciplukan stem extract test, the leaf extract of ciplukan with chloroform solvent showed the best inhibition zone with a concentration of 5% and extremely different effects with concentrations of 10%, 15 %, and 20%, as shown in the table below.

Table 2. Results of analysis of variance of inhibition zone of ciplukan leaf extract with chloroform solvent on Vibrio spp bacterial isolates from intestine of vannamei infected by WFD.

| Treatment of Extract concentration | Average of inhibition zone (mm) | Notation (p>0.01) |
|-----------------------------------|---------------------------------|-------------------|
| 0%                                | 6                               | A                 |
| 5%                                | 10.25                           | B                 |
| 10%                               | 13                              | C                 |
| 20%                               | 13                              | C                 |
| 15%                               | 14.5                            | C                 |

To determine the effect of ciplukan extract with different solvents, a variance analysis of ciplukan extract with chloroform and ethyl acetate solvents was carried out which gave the highest inhibitory effect on antibacterial tests. The results of variance analysis illustrated that at the same concentration, extracts with different solvents had a very significant effect on the inhibition zone on the growth of Vibrio spp. bacteria. The leaf extract of ciplukan with ethyl acetate solvent gave the lowest inhibition zone (8 mm) at the identical concentration. Meanwhile, the stem extract of ciplukan with ethyl acetate solvent has a higher inhibitory capacity of 12 mm and is not significantly different from leaf extract of ciplukan with chloroform solvent and stem extract of ciplukan with chloroform solvent.

Table 3. Results of variance analysis of inhibition zone of ciplukan extract with different solvents against Vibrio spp. bacterial isolates from intestine of vannamei shrimp infected by WFD.

| Treatment Kinds of Extracts | Average Inhibitory Zone (mm) | Notation (p>0.01) |
|-----------------------------|------------------------------|-------------------|
| Stems of ciplukan Chloroform| 13                           | A                 |
| Leaves of ciplukan Chloroform| 13                           | A                 |
| Stems of ciplukan ethyl acetate | 12                           | A                 |
| Leaves of ciplukan Acetate | 8                            | B                 |

Based on the result of the analysis, the extract used for the following test was ciplukan extract with chloroform solvent, both from the stem and leaves, on the isolate of green bacteria Vibrio colonies and yellow colonies as the result of the intestine as well as the water of shrimp culture infected by WFD.
3.3. Toxicity test of ciplukan extract
The survival of Vannamei shrimp after soaking in ciplukan chloroform extract once for one hour showed that 50% concentration of ciplukan chloroform extract had a toxic effect on vannamei shrimp is 50%. Meanwhile, lower concentrations are relatively non-toxic since the obtained survival is still ranging from 83 to 100%. Thus it can be concluded that ciplukan chloroform extract is secure to utilize as an antibacterial substance for shrimp if the concentration is lower than 25% with soaking period for one hour or 25% concentration with soaking period for less than an hour.

4. Conclusion
In conclusion, the results of this study reveal that ciplukan herbs contain active steroid substances, triterpenoids, tannins, flavonoids, alkaloids. Extracts with chloroform and ethyl acetate solvents have the highest antibacterial activity with 16.5 mm inhibition zone. The best concentration that provides strong inhibitory zone is 10%, which results in a 13 mm zone of inhibition and does not differ with concentrations of 15 and 20%. Ciplukan herbal extract is toxic at a concentration of 50%.

5. References
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