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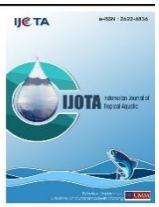

Evaluation of cherry leaf extract on sangkuriang catfish against *Trichodina* sp

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ABSTRACT

The aimed of this study is to evaluate of cherry leaf extract *(Muntingia calabura)* on hematocrit, leukocrit and anti-parasitic on *s*angkuriang catfish *(Clarias gariepinus)* post-infected by *Trichodina* sp. Cultivators experience constraints on seeding and enlargement of ectoparasites *Trichodina* sp. This parasite infected on the surface body with appear spots on the body. Prevention and treatment using many chemicals generated adverse effect such as residue, and environmental hazard. In other hand, Cherry Leaf contains Flavonoid, Tannin, Triterpenoid, Saponin and Polifenol compounds as antioxidants and antibacterial. This research used experimental method and Completely Randomized Design (RAL). Concentrations of cherry leaf extract *(Muntingia calabura)* were devided in 1%, 2%, 3%, 4% and 5% applied on 5 treatments and 4 replications. The data were analyzed using ANOVA method *(variance analysis)*. The results revealed cherry leaf extract *(Muntingia calabura)* very good against *Trichodina* sp parasite. The best value of the leukocrit percentage showed by 2% concentration that decreased leukocrit percentage to 2.68% and increased percentage of hematocrit was increased 11.83% post-infected. The clinical study, funded white spots come out in all body, pale color appearance, decreased appetite and rubbing the body to the wall. Water quality measurement showed temperature: 24 - 27,2^{o}C, DO 4,1 - 6,9 and pH: 6 – 8.

Keywords: cherry leaf, Hematocrit, Leukocrit, Sangkuriang Catfish, *Trichodina*

1. Introduction.

Sangkuriang Catfish *(C. gariepinus)* become the leading commodity of Indonesian freshwater fishery. It is one source of animal proteins contributed for the nutritional needs of Indonesian society. In the present, the farmers still have a problem in the hatchery and enlargement phase of Sangkuriang catfish (*C. gariepinus*). The hatchery phase have been known a vulnerable phase on disease invasion. The *Trichodiniasis* caused by *Trichodina* sp, one of the diseases, is often infected of freshwater fish included *C. gariepinus* seeds.

Trichodina sp, an ectoparasite and ciliate parasite, infect on the surface of the skin, fins and gill that is caused decreasing of immune system and generates secondary infection (Zheila, 2013). It infects by sticking to the epithelial layer of the fish with the hook. The hook destroys epithelial cells by twisting, then damaged epithelial cells (Yuasa, 2003). According to Gusrina (2008),

Trichodina sp is characterized by the presence of white spots to gray and remove mucus. Characteristics of *Clarias* sp infected by *Trichodina* sp were looked dull color, visible limp and often rub their body against the wall or bottom of the pool.

Chemicals and antibiotics such as CuSO4 and Formalin have been used to prevent and treat of *Trichodina* sp infection (Mahasri, 2009). The use of chemicals such as formalin cause cancer for consumers, eye and skin irritation and respiratory disorders (Chanif, 2012). Based on SNI 01-6729-2002, natural ingredients could be an alternative to prevent of infection. The use of natural ingredients can reduce the residue and does not affect the environment and host. One of the materials that can be used is Leaf of Cherry *(M. calabura)*.

Priharyanti, (2007) and Zakaria, (2007), Cherry Leaf contains Flavonoid, Tannin, Triterpene, Saponin and Polifenol as antioxidants. Cherry Leaf is also useful as an anti-bacterial (Sulistyaningrum, 2014). Polar compounds in Saponin, Flavonoid, and Tanin are active as antimicrobials by destroying cytoplasmic membranes and killing epidermal cells (Rahayu, 2008). This study was conducted to examine the effect of *M. Calabura* extract as anti-parasitic agent against *Trichodina* sp on Sangkuriang catfish *(C. gariepinus)*.

2. Materials and Methods

The study was conducted in May - June 2017 at Fisheries Laboratory, Faculty of Animal Husbandry and Chemistry Laboratory of University of Muhammadiyah Malang. The research material was Sangkuriang catfish, cherry leaf, ethanol 96%, FeCL3 1%, HCL 2 N, concentrated H2SO4, concentrated H2SO4, Bouchartdat, and Dragendof, concentrated HCl, Mg powder, Amyl alcohol, Filter paper, Catfish feed, NaCl Physiological, EDTA, Plastic Candle, Universal Indicator. The research tool was Aquarium, blower, scales, filter, microscope, glass concave object, DO meter, Eppendorf tube, hematocrit tube, syringe, rotatory evaporator, oven, Erlenmeyer, beaker glass, pumpkin, spatula, measuring pipette, blander, , water stone, aeration hose, aeration faucet, paralon pipe, LED lamp, small tub, sectio set, thermometer, centrifuge. This study used the experimental method with Completely Randomized Design (RAL) because the experimental media was considered homogeneous. The Completely Randomized Design Model used is: Yij = μ + αi + εij. The design was used 5 treatments and 4 replicates ((1%, 2%, 3%, 4% and 5%) and controls}.

2.1. Research procedure

This research included extraction of cherry leaf, Phytochemical Extract Test, Solution Preparation Test, Parasite Preparation Test, Container Preparation Test, Soaking of Sangkuriang Catfish Seed in Cherry Leaf Extract and Hematocrit and Leukocrit Testing.

2.2. Static Analysis

Data analysis on the hematocrit and Leukocrit levels used the analysis of variance and the Smallest Differential Difference Test (BNT) to compare values between treatments. Description data was analyzed from a clinical symptom, microscope observation, and water quality.

3. Results and Discussion

3.1. Results

3.1.1. Extraction

4.5 kg of Fresh *M. calabura* cherry leaves could be extracted to be 1.25 kg of yield powder. Maceration results obtained 935 ml then evaporated to 720 ml that employed 96% ethanol with 4 liters/kg of powder of cherry leaves, in ratio.

3.1.2. Phytochemical test

Phytochemical test was applied to identify cherry leaf compounds.

Table 1. Phytochemical test results of Cherry Leaf *(Muntingia calabura)*

Test	Results	Description
Alkaloids	1. Bening (Mayer Test)	(–)
	2. Bening (Test Bouchardat)	(–)
	3. Brownish brown sediment (Dragendorf Test)	(+)
Flavonoids	The orange layer on amyl alcohol	(+)
Tanin	The color is blackish green	(+)
Saponin	There is a permanent foam on the top layer	(+)
Steroid/ Terpenoid	Shaped green	(+)/(–)

3.1.3. Leukocrit

Leukocrit was calculated to know the percentage of leukocyte content in sangkuriang catfish seeds infected by *Trichodina* sp pre- and post-immersion of cherry leaf extract. The results (Fig. 1) showed that cherry leaf extract *(M. calabura)* worked well against *Trichodiniasis* disease on sangkuriang catfish *(C. gariepinus)*. It showed significant difference among treatment (p<0.05). it revealed that the leukocrit treatment value of 1% was Pretreatment 20.15% and Post Treatment to 3.44%, 2% treatment was Pretreatment 24.39% and Post Treatment to 2.68%, 3% treatment was Pretreatment 24, 68% and Post Treatment to 4.48%, 4% treatment was Pretreatment 23.52% and Post Treatment to 7.56%, treatment 5% was Pretreatment 18.77% and Post Treatment to 5.77%. Based on results, it showed that 4% of cherry leaf extract had the highest leukokrit percentage of Sangkuriang catfish seeds with 7.56%, while the lowest percentage of leukokrit content was in 2% treatment with 2.68 % level of leukokrit.

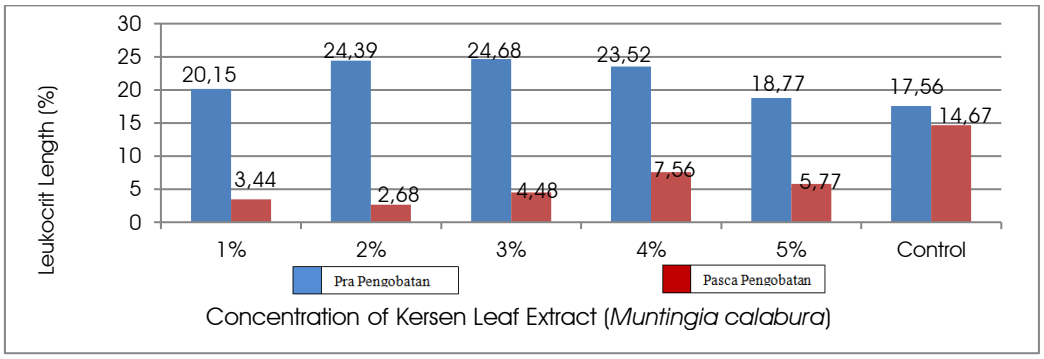


Figure 1. Percentage of Leukocrit Value

3.1.4. Hematocrit

Hematocrit was measured to see the percentage of erythrocyte contents in catfish seed infested by *Trichodina* sp parasite post-immersion with cherry leaf extract. The percentage of hematocrit was observed in pre- and post-treatment.

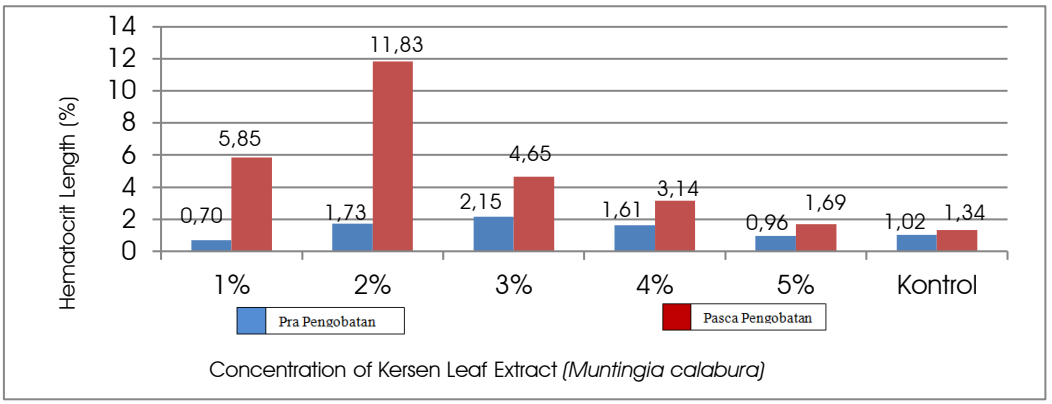


Figure 2. Percentage of Hematocrit Value

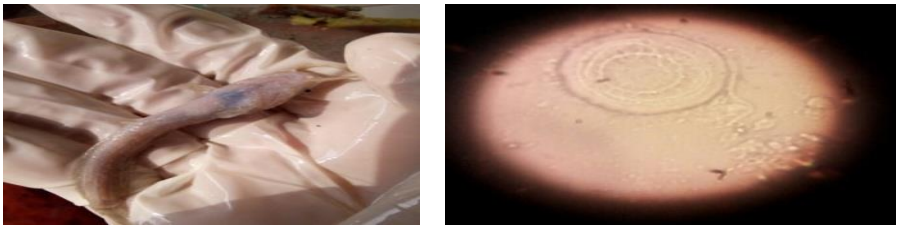
The results of variance showed cherry leaf extract *(M. calabura)* as the treatment of *Trichodiniasis* disease significant different (p<0.05) on sangkuriang catfish seeds *(C gariepinus*).

Based on fig. 2, showed that ematocrit values of treatment 1% were pre-treatment 0.70% and post-treatment to 5.85%, 2% treatment was 1.73% and post-treatment treatment became 11.83%, treatment 3% was pre-treatment 2.15% post-treatment to 4.65%, 4% treatment was 1.61% pretreatment to 3.14% post-treatment, 5% treatment was pre-treatment 0.96% and post-treatment to 1.69% and pre-treatment control 1, 02% and post-treatment 1.34%. Based on our results, it showed that 2% of cherry leaf extract the highest of hematocrit percentage of catfish seed with 11.83%, then the lowest percentage of hematocrit content was at 5% by 1.69% level of hematocrit.

3.1.5. Clinical Symptoms

Clinical symptoms were applied to determine the specific conditions in the observed biota. The control group changed post-infected with wounds appeared in throughout body, abnormal swimming, many mucus and was given with a symbol (+++). The 1% and 2% of *M. calabura* extract showed clinical changes on 18th and 17th day, respectively (++) and the 7th day ((+)) and (+) at 19th day. The 3% concentration of *M. calabura* extract showed a better results. The clinical symptoms changed on 18th day with the symbol (++), on the 90th day with the symbol (+) and on the 20th day with the symbol ((+)). At the 4% concentration, it changed on the 19th days with symbol (++) and symbol ((+)) on the 20th day. The 5% concentration changed on the 19th days with symbol (++) and on a day to twenty with symbols (++) and ((+)).

The seeds of catfish was changed morphologically and physiologically post-infected by *Trichodina* sp. It was marked by the presence of white spots on the catfish body, excessive of mucus and rubbed their body on the wall. Infection of *trichodina* parasite could cause death in fish (host).



(a) (b)

Figure 3. (a) Trichodina observation is macroscopic and (b) Microscopic

3.2. Discussion

The immersion *(maceration)* was employed to obtain cherry leaf extract. According to Agoes (2007), maceration is a simple way of filtering. The solvent will penetrate into cell wall and cell cavity contained an active substance. The active compound will be dissolved because there is a difference of concentration between the active substance inside and outside cell. According to Krisdayanti (2011) and Kuntorini, et al. (2013), cherry leaf consists of dry material such as crude protein, crude oil, ether extract, neutral detergent fiber, acid detergent fiber, tannin compounds, flavonoids, and saponins. The extract was obtained 720 ml using evaporation process. The ethanol was used to extract antimicrobial compounds from plants which are saturated aromatic and organic compounds. Based on Watunglawar (2013), ethanol had been employed to extract compound of cherry leaf extract *(M. calabura L)* to inhibit *Vibrio harveyi*. According to the description above, cucumber leaf extract was recommendable applied to treat *Trichodina* sp parasite on cultivation.

3.2.1. Leukocrit

Leukocrit value of catfish is 1-2% in normal condition. Leukocrit levels are less than 1% in chronic infection such as low nutritional quality, vitamin deficiency, and contaminants. Leukocrit levels are more than 2%, probably due to early stage of infection and stress in catfish (Perera & Pathiratne, 2008 in Husayin 2016). The results showed decreasing of leukokrit by all treatment in all time-manner. The 2% of cherry leaf extract showed the best effect to decrease catfish leukokrit compared among treatments ($p>0.05$). Decreasing of leukocrit percentage in this study is revealed that cherry leaf extract could inhibit *Trichodina* parasite infection. We speculate that is related to the content of active substances contained in cherry leaf extract (Table 1) that have been known as antioxidant, anti-bacterial and anti-microbial compound (Krisdayanti, 2011 and Kuntorini et al., 2013).

Flavonoids are phenol compounds that are disinfectant works by denaturing proteins that can cause microbial cell metabolic activity to stop because all microbial cell metabolism activity is catalyzed by an enzyme that is a protein. The cessation of this metabolic activity can lead to death in microbial cells. Flavonoids are also bacteriostatic which works by inhibition of bacterial cell wall synthesis (Selway, 1986). According to Sabir (2005), flavonoid compounds have the ability to inhibit the growth of bacteria with several different mechanisms, including flavonoids causing damage to the permeability of bacterial walls, microsomes, and lysosomes as a result of the interaction between flavonoids with bacterial DNA, different mechanisms proposed by In Carlo *et al.,* (1995) and Estrela *et al.,* (1995) in Sabir (2005), which states that hydroxyl groups present in the structure of flavonoid compounds cause changes in organic components and nutrient transport that will eventually lead to toxic effects on bacteria.

Cherry leaf extract *(M. calabura)* also contain tannin, which has ability to be antibacterial from proteins complexes and bind to bacterial cell walls to inhibit bacterial growth or enzyme activity (Smith et al., 2005). Other Cherry leaf *(M. calabura)* compound is saponin. Saponin is a strong active compound and could cause foam and has the ability as toxic to aquatic (fish). In other case, saponin compounds have the antibacterial ability with increase the permeability of bacterial cell membranes caused damage and lysis of cell (Robinson, 1995). Furthermore, Dwidjoseputro, (1994) suggests that saponins have molecules that can attract water or hydrophilic and molecules that can dissolve fat or lipophilic that can reduce the surface tension of cells that ultimately lead to the destruction of germs.

3.2.2. Hematocrit

Increasing of hematocrit level may indicate a contaminant, problem in osmolarity and stress. Decreasing of hematocrit levels indicate contamination condition such as lack of food, vitamin deficiency or infection, while in normal hematocrit values is ranged from 20-45% (Husin, 2016). The results showed increasing of hematocrit by all treatment in all time-manner. The highest increasing of catfish hematocrit level was showed by 2% of cherry leaf extract compared among treatments ($p>0.05$). Increasing of hematocrit percentage in this study revealed that cherry leaf extract could inhibit *Trichodina* parasite infection.

Increasing of hematocrit is believed because of immersion of cherry leaf extract. Although the percentage of the hematocrit is not in normal range, it is possible because it is still in the recovery phase. According to Hussein, (2016) Hematocrit levels are influenced by the initial condition and handling of fish such as sampling fish blood. It could decrease of fish hematocrit level. In other case, Fish also has a anemia which make a percentage of hematocrit about 10%. The low hematocrit level indicate contamination, lack of food, the low protein content of feed, vitamin deficiency or the occurrence of infection. The high of hematocrit may also show contamination, osmoregulation and stress problems (Hastuti, 2007). Fujaya (2004) in Hastuti (2007), there is a strong relationship between hematocrit and the amount of blood hemoglobin. The lower of red blood cells as same as the lower the hemoglobin blood contents.

Based our results, increasing of hematocrit values might be caused by active substances contained in cayenne leaf extract. Kuntorini et al*.,* (2013) revealed that the content of cherry leaf compounds are flavonoids, saponins, triterpenoids, steroids, and tannins showed antioxidative activity. In previous studies suggested that those compounds are antioxidant, anti-bacterial and anti-microbial which at certain concentrations could disturb fish physiologically because of the endurance of fish to the extract. According to Sabir (2005), flavonoid compounds have ability to inhibit bacteria growth with different mechanisms, including flavonoids causing damage to the permeability of bacterial walls, microsomes, and lysosomes as a result of interactions between flavonoids and bacterial DNA.

3.2.3. Clinical Symptoms

Trichodina sp parasites interfere in freshwater activities. *Trichodina* sp parasite adhere in the skin, fins, and gills. The Fish infected by *Trichodina* sp become pale, decrease of appetite, slow motion, often rubbed on the wall, and irritation. Other clinical symptoms, white spots appear on the head and back and grow because of excessive mucus production (Khordi, 2010). *Trichodiniasis* in some cases cause several damage to the host, which lead to host mortality (Woo, 2006). The infection of *Trichodina* sp could be faster when the water condition is fit with their physiology. the level of pathogenicity is the ability to infect *Trichodina* sp is the prevalence and intensity (Irianto, 2005).

According to Woo (2006) and Basson (2010), *Trichodina* sp could be multiply by splitting rapidly and always moving actively to increase invasion intensity. The type and extent of *Trichodina* sp infection could be different because they affect in different area and stage of fish such as in feed, fish age, fish size, aquatic conditions and cultivation activities (Handayani et al., 2014). Based on Hadiroseyani *et al.,* (2006), the high density of stocking will cause fish to touch each other which facilitate a transmission. The clinical symptoms of fish usually appear because of excessive mucus production and weak in behavior, skin lesions and fins slightly damaged. Parasites in large quantities in the gills could disturb fish breathing and damage gill epithelium. High mortality generally is occured in small fish (Ansari, 2008). According to Pramono and Hamdan (2008), *Trichodina* sp

parasites in high intensity could damage to the gill structure and leads to death. Actually, this parasite is not the main pathogen because they infect fish after other factors appear such as injury, pain, and stress. This parasite makes the fish body as a sticky place (substrate) and takes organic particles from bacteria attached to the skin of fish caused wounds. Attachment to the gills cause injuries and frequent red blood cells in the *Trichodina* sp food vacuole. In this condition, *Trichodina* sp is a true ectoparasite (Rahayu, 2009).

3.2.4. Water quality

Water is a habitat for aquatic animals especially fish, therefore water parameters are kept within the optimum range that could support the life and growth of fish. Sangkuriang catfish is a type of fresh fish whose is influenced by environmental factors. In this research, water quality measurements were observed such as temperature, pH, and DO. These parameters were observed every morning/day.

Table 2 Parameter Range Water quality at the time of study

Treatment	Parameter		
	Temperature °C	pH	DO (ppm)
Control	24-27,2	6-8	4,1-6,3
Treatment	24,27	6-8	4,1-6,9

Based on table 2, it was known that the temperature in range from 24 - 27.2 °C, DO (Dissolved Oxygen) range from 4.1 - 6.9 ppm and the pH of water in range from 6-8. The quality of water during the study was still in accordance and feasible for the life of catfish. According to Suryaningsih, (2014) catfish could live optimally on the DO range of 4 mg/liter, pH range 6-8 and temperatures ranging from 26-29°C.

4. Conclusions

Cherry Leaf Extract *(M. calabura)* has an effect to treat *Trichodiniasis* disease revealed by decreasing value of Leukocrit presentation on blood of Sangkuriang catfish, increasing of the percentage of Hematocrit and body condition of Sangkuriang catfish begin to normal (healthy). The 2% concentration is the optimal concentration to treat *Trichodina* sp parasite, with the value of Leukocrit percentage of 24.39% post- infected and to 2.68% post-treatment. The percentage value of Hematocrit was 1.73% post- infected and to 11.83% post-treatment.

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