Correlation of Concentration Ethyl Acetate Fraction of Calabash Fruit (*Crescentia cujete* L.) with Mortality *Haemonchus contortus* Worm In Vitro

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Abstract

The purpose of this study was to ascertain how the concentration of the ethyl acetate component of calabash fruit (*Crescentia cujete* L.) affected the in vitro mortality of the *Haemonchus contortus* worm. This study employed a post-test only control group design as its methodology. There were five treatments, with a total of four repetitions. Twenty *H. contortus* worms were utilized as samples in each treatment throughout all replications. Observation and recording of *H. contortus* mortality was carried out at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 minutes, and when all worms in the petri dish died. Worm mortality was observed by examining whether there was a movement reaction from the worms when given a touch or water droplets at 50°C. Linear regression test was used to assess the data. The resulting linear equation, y = 2.417 + 5.875x, demonstrated that the ethyl acetate component of calabash fruit had an impact on worm mortality. The conclusion of this study is that the increase in the concentration of the ethyl acetate fraction is directly proportional to the mortality of the worm *Haemonchus contortus* in vitro with an effect value of 93.4%.

Keywords: *Haemonchus contortus,* ethyl acetate fraction of calabash fruit, linear regression test

Introduction

Haemonchosis is a digestive tract disease caused by worms of the genus *Haemonchus*. One species of worm that is often found and causes this disease in Indonesia is *Haemonchus contortus* with a predilection for the abomasum of goats and sheep (Noviana *et al*., 2017). Intestinal nematode worm infection in small ruminant animals is dominated by *H. contortus*, with a frequency of 58.26% - 66.21%, according to research by Nugroho (2013). The incidence and infection rate of the deadly parasite *H. contortus* can approach 80%, and it can kill goats in as many as 66.7% of cases (Suteky and Dwatmadji, 2010). Haemonchosis is 89.4% prevalent in Indonesia, according to FAO (1991). According to Nugroho (2013), *H. contortus* was the most common cause of haemonchosis in Subang District, Banyumas, which has a 58.26–66.21% frequency**.** Mariyam *et al*. (2018) reported the prevalence of *H. contortus* in Pegirian RPH Surabaya in September-November 2017 of 47.2%. According to Arifin *et al*. (2019), the rate of haemonchosis in Banyuwangi's Kalipuro District from January to February 2019 was 15%. If serious control efforts are not done, this worm will inflict losses of up to 7.000.000/year (Rachmat *et al*., 1998 and Ahmad *et al*., 2006).

Giving synthetic worm medication is how this condition is typically controlled. However, during administration, it is frequently mishandled and leads to drug resistance (Fitri *et al*., 2011), hence it is important to create and find plant-based medications (herbal medicines). Secondary metabolites of flavonoids, alkaloids, and tannins in herbal plants can function as anthelmintics, according to Sumual *et al*. (2021) and Rahayu *et al*. (2021).

The tropical plant species known as calabash (*Crescentia cujete* L.) is a member of the Bignoniaceae family (Mahbud *et al*., 2011). Indonesia, which is still a part of Southeast, has the ability to produce calabash plants, according to Rismayani (2013). Although this plant is easily accessible and ubiquitous throughout Indonesia, its application is still uncommon (Bahroni and Istianah, 2017). Phytochemical screening of the

ethyl acetate fraction of calabash fruit that has been carried out by Billacura and Laciapag (2017) proves that the ethyl acetate fraction of calabash fruit contains tannins, flavonoids, alkaloids, and can be used as anthelmintics in *Eudrilus eugenia* worms.

Method

This study uses a post-test only control group design in a genuine experimental setting with a completely randomized design (CRD). The worm *H. contortus* from a goat abomasum that had been rejected served as the study's sample. It was collected from the Pegirian Slaughterhouse, Surabaya. In order to survive outside the host body, twenty *H. contortus* worms with criteria still actively moving were placed in a container containing 0.9% physiological NaCl solution (Jeyathilakan *et al*., 2010) in this study's five treatments, each of which consisted of four replications.

Petri dishes, pipettes, tweezers, stirring rods, water baths, incubators, 500 mL beakers, 100 mL measuring cups, digital scales, thermometers, gloves, masks, extraction tools, fractionation, and stopwatches were the equipment utilized in this work. Calabash fruit, aquades, 0.9% physiological NaCl solution, tween 80, 96% ethanol, ethyl acetate, levamisole, and *H. contortus* worms were the ingredients employed in this investigation.

Calabash Fruit Ethyl Acetate Fraction Preparation

Fruit from calabash was dried, ground into a powder, and macerated with 96% ethanol. The residue is macerated one more till it turns brown after the maceration results are filtered. At a temperature of 40–50 °C, the resulting filtrate was mixed and concentrated until a thick extract was produced (Khoirani, 2013). With ethyl acetate serving as the solvent, the resultant viscous extract was segregated. The filtrate was evaporated using a rotary evaporator to get the ethyl acetate fraction of calabash fruit (Sudarmika *et al*., 2021).

Preparation of Calabash Fruit Ethyl Acetate Fraction in Various Concentrations

Concentration was made using the formula (w/v) which refers to the research of Billacura and Laciapag (2017) with modifications obtained from preliminary research, namely the ethyl acetate fraction of calabash fruit that has been made taken as much as each 0.025; 0.05; and 0.1 grams and 1% Tween 80 then added physiological NaCl solution to a volume of 20 mL to obtain concentrations of 0.125%, 0.25%, and 0.5%.

Making the Levamisole Solution

Levamisole Oral Anthelmin powder was used to create the levamisole solution that was utilized as a positive control. A concentration of 10 mg/mL was necessary for this study (Billacura and Laciapag, 2017). In this study, 0.2 grams of Anthelmin Oral Levamisole were required, and it was dissolved in 20 mL of physiological NaCl and 1% Tween 80.

Data Analysis

The linear regression test was used to investigate the association between the concentration of the ethyl acetate fraction and the quantity of worm mortality.

Result and Discussion

The results of this study were the data of the number and the duration of worm deaths. The average and standard deviation of the percentage of worm mortality will be determined based on mortality statistics, as shown in Table 1.

The data obtained were analyzed using a linear regression test to predict the number of worm deaths that were influenced by the concentration of the ethyl acetate fraction of the calabash fruit. From the results of the linear regression test output, it can be seen that the correlation coefficient of the concentration variable with the number of mortality shows the number is positive (r = +0.996), which means the correlation coefficient of the concentration variable with the variable number of worm mortality is (r = +0.966), which is positive which indicates a positive correlation between them, it indicates that more extraction concentration (ethyl acetate fraction) affects more worm mortality. The 2-tailed sig is 0.000 which has the following interpretation: The probability value or 2-tailed sig shows the number 0.000 less than 0.01, which means that there is a hightly significant ($p<0.01$) correlation between the concentration variable and the number of worm mortality variables.

Table 1. Average and Standard Deviation Percentage of *H. contortus* Worms that Died Every 5 Minutes After Treatment in All Treatments

Treatment	Observation Time (Minutes)											
	5	10	15	20	25	30	35	40	45	50	55	60
K-	0,00^{a} ± 0,00	0,00^{a} ± 0,00	0,00^{a} ± 0,00	0,00^{a} ± 0,00	0,00^{a} ± 0,00	0,00^{a} ± 0,00	0,00^{a} ± 0,00	0,00^{a} ± 0,00	0,00^{a} ± 0,00	0,00^{a} ± 0,00	1,25^{a} ± 2,50	1,25^{a} ± 2,50
K+	23,75^{c} ± 4,78	58,75^{d} ± 13,76	73,75^{d} ± 14,93	82,50^{e} ± 6,45	88,75^{e} ± 4,78	98,75^{d} ± 2,50	100,00^{d} ± 0,00	100,00^{d} ± 0,00	100,00^{d} ± 0,00	100,00^{d} ± 0,00	100,00^{d} ± 0,00	100,00^{d} ± 0,00
P1	0,00^{a} ± 0,00	1,25^{ab} ± 2,50	2,50^{a} ± 2,88	7,50^{b} ± 6,45	11,20^{b} ± 4,71	15,00^{b} ± 9,12	22,50^{b} ± 13,22	28,75^{b} ± 16,52	32,50^{b} ± 15,54	36,25^{b} ± 12,47	38,75^{b} ± 11,08	41,25^{b} ± 11,08
P2	1,25^{a} ± 2,50	6,25^{b} ± 6,29	8,75^{b} ± 4,78	17,50^{c} ± 2,88	26,25^{c} ± 8,53	35,00^{c} ± 9,12	43,75^{c} ± 14,93	53,75^{c} ± 11,81	58,75^{c} ± 8,53	63,75^{c} ± 11,08	68,75^{c} ± 7,50	71,25^{c} ± 6,29
P3	10,00^{b} ± 4,08	22,50^{c} ± 10,40	32,50^{c} ± 11,90	55,00^{d} ± 10,80	71,25^{d} ± 13,15	82,50^{d} ± 15,54	88,75^{d} ± 8,53	93,75^{d} ± 6,29	100,00^{d} ± 0,00	100,00^{d} ± 0,00	100,00^{d} ± 0,00	100,00^{d} ± 0,00

Note: There were significant variations between the lowercase superscripts ($^{a\ b\ c\ d\ e}$) in the same column ($p<0.01$)

In the "Model Summary" table, which can be read in the R square box, the value is 0.934 which means that the effect of the concentration variable on the variable number of worm mortality is 93.4% while 6.6% is influenced by other variables besides the concentration variable.

In the table the coefficients obtained in column B at constant (a) are worth 2.417 and at concentration (b) are valued at 5.875. The R value in the linear regression results in this study was 0.966, which could be concluded that the concentration of the ethyl acetate fraction of fruit calabash (*Crescentia cujete* L.) had a strong relationship in influencing the number of worm mortality, while the R^2 value was 0.934. From the calculation results obtained the regression equation, namely:

y : a + bx
: 2,417 + 5,875x
y : Total worm mortality
x : Concentration of ethyl acetate fraction of calabash fruit

The linear regression equation above can be presented in graphical form as in Figure 1.

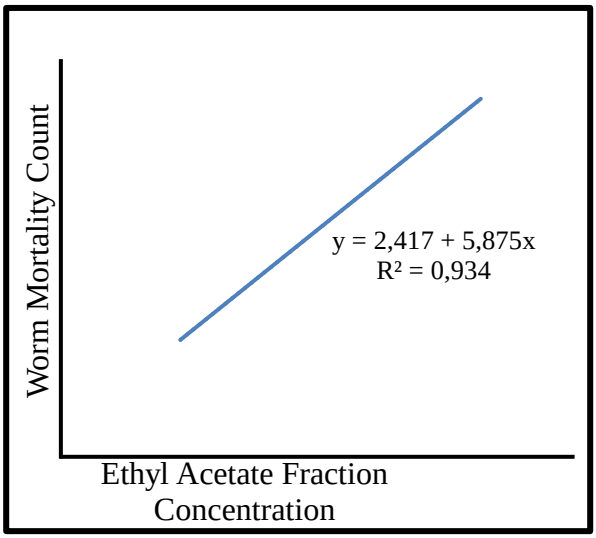

Figure 1. Graph of the linear regression equation between the concentration of ethyl acetate fraction in calabash fruit and *H. contortus* worm mortality.

Based on Figure 1, it is clear that the regression equation's straight line points upward, indicating a direct correlation between the concentration of ethyl acetate in calabash fruit (*Crescentia cujete* L.) and the death of *H.*

contortus worms. This suggests that worm mortality is increasing at a quicker rate when the concentration of the ethyl acetate fraction of calabash fruit increases.

The Research and Industrial Consultancy Institute in Surabaya conducted phytochemical studies, and the results showed that the calabash fruit contains alkaloids, tannins, and flavonoid chemicals. If the observation of worm mortality is related to the test of the active substance contained in it, then the anthelmintic power of the ethyl acetate fraction of calabash fruit comes from the content of the active substance. When flavonoids are used as anthelmintics, they work by producing protein denaturation in worm tissue, which kills the worms (Faradila, 2013). According to Patel *et al.* (2010), tannins function as anthelmintics by impeding the oxidative phosphorylation process, which results in death, while alkaloids have the same function as saponins, namely inhibiting the ability of the cholinesterase enzyme to break down acetylcholine, a neurotransmitter that carries nerve impulses (Sandika *et al*., 2012). Therefore, the greater the concentration of the ethyl acetate fraction of calabash fruit, the more active substances it contains in causing the death of the worm *H. contortus*.

Conclusion

The increase in the concentration of the ethyl acetate fraction of calabash fruit is directly proportional to the mortality of *H. contortus* worms, so that the increase in the number of worms' mortality caused is greater as the concentration of the ethyl acetate fraction of worms is given.

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