



Effectiveness of *Crescentia cujete* Linnaeus Fruit Ethanol Extract as *Aedes aegypti* Linnaeus Larvicidal

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Abstract

Dengue fever vector control currently uses chemical larvicides, but the use of chemical larvicides is considered less effective because *Ae. aegypti* larvae have developed resistance. Berenuk plant (*C. cujete*) is one of the plants that has potential as a larvicide. The aim of the research was to analyze the effectiveness of *C. cujete* fruit skin extract and flesh extract as an *Aedes aegypti* larvicide and to determine the content of active compounds found in the skin and flesh of berenuk fruit. Phytochemical screening of fruit skin extract and fruit flesh extract using thin layer chromatography test. Mortality analysis of *Ae. aegypti* larvae using Two-way Anova and probit to determine the LC50 value. Research results show that *C. cujete* fruit skin and flesh extract contained alkaloid, flavonoid and triterpenoid compounds. *C. cujete* fruit skin extract and flesh extract can cause mortality of *Ae. aegypti* larvae with LC50 values, namely the concentration of fruit skin extract 3415.007 ppm and fruit pulp extract 3112.767 ppm during 96 hours of observation. Fruit skin extract and fruit flesh extract of *C. cujete* have larvicidal effect against *Ae. aegypti*.

Keywords: *Aedes aegypti*, *Crescentia cujete*, Larvicide, Thin layer chromatography

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Introduction

Dengue fever cases in Indonesia are observed to increase when entering the transition period from the dry season to the rainy season. Based on records from Direktorat Pencegahan dan Pengendalian Penyakit Menular (P2PM) until the 36th week, the number of confirmed cases of dengue fever in 2022 was reported as 87,501 cases with 816 deaths. Districts/cities that recorded the highest dengue fever cases include Bandung City with 4,196 cases, Bandung Regency with 2,777 cases, Bekasi City with 2,059 cases, Sumedang Regency with 1,647 cases and Tasikmalaya City with 1542 cases reported (Kementrian Kesehatan Republik Indonesia, 2022).

Kementerian Kesehatan Republik Indonesia stated that dengue fever is commonly found during the rainy season. This is because the dengue fever vector reproduces a lot in the rainy season. Dengue fever is caused by the dengue virus which is spread through vectors, especially the *Aedes aegypti* L. mosquito

(Menkes RI, 2020). Based on the mode of infection, dengue fever prevention and control are focused on breaking the chain of the mosquito life cycle by implementing vector control (Adrianto et al., 2018).

Dengue fever can be minimized by controlling the population and spread of the mosquito vector *Ae. aegypti*. Dengue vector control is generally using chemical larvicides such as Temephos. Chemical larvacides became part of the dengue vector mass extermination program established in 1980 and still applied today (Cahyati et al., 2019). However, the effectiveness of temephos may be reduced by the development of insecticide resistance in *Ae. aegypti* populations. Recent studies have reported resistance to temephos in several regions of Indonesia, highlighting the need for continuous resistance monitoring and integrated vector management (Ridha et al., 2025; Sofiana et al., 2023; Zulfa et al., 2022). Temephos is an organophosphate larvicide that primarily acts on the cholinergic system through inhibition of acetylcholinesterase. In addition to concerns regarding resistance, previous toxicological

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evidence has reported potential adverse effects of temephos exposure on several biological systems, including the nervous system, liver, and male reproductive system (Martínez-Mercado et al., 2022; Sabri et al., 2024). Therefore, the prolonged and repeated use of temephos should be carefully monitored to maintain its effectiveness and minimize potential risks to human health and the environment. These limitations emphasize the need to investigate alternative or complementary larvicidal agents that can support existing chemical based vector control strategies.

Plant derived larvicides have received increasing attention as potential alternatives to conventional synthetic insecticides because their bioactive constituents may exhibit larvicidal activity and may offer advantages related to biodegradability and environmental persistence. Recent reviews have highlighted the potential of plant-based extracts for mosquito control as environmentally friendly alternatives to synthetic insecticides (Hillary et al., 2024; Noorazlan et al., 2023). However, the use of botanical extracts should not automatically be considered safe for humans or the environment, because their toxicity can vary according to the plant species, plant part, extraction solvent, concentration, and target or nontarget organism. Therefore, safety assessment, particularly against nontarget organisms and under environmentally relevant conditions, remains necessary before practical application.

Crescentia cujete is a potential candidate for investigation as a botanical larvicide because its fruit contains various secondary metabolites, including alkaloids, flavonoids, tannins, and saponins, which may contribute to its biological activity. Based on these characteristics, the evaluation of *C. cujete* fruit skin and flesh as potential sources of botanical larvicides may provide an alternative approach for *Ae. aegypti* control. Nevertheless, the present study does not assume that *C. cujete* extract is inherently safe for humans or the environment. Rather, the study focuses on evaluating its larvicidal activity against *Ae. aegypti*, while further studies on toxicity to non-target organisms, biodegradability, environmental persistence, and safety are required to establish its suitability for practical application.

The World Health Organization (WHO) recommends the development of natural vector control that is environmentally friendly and safer for human health. Breaking the chain of disease infection with chemical larvacides can be replaced with natural larvicides (Handayani et al., 2018). Natural larvicides from plant raw materials have been proven as a new alternative to reduce mosquito density as a vector (Riyadi et al., 2018). The effect as a larvicide is obtained from secondary metabolite compounds contained in plants which include alkaloid, flavonoid, tannin and saponin compounds (Marini et al., 2018). There are several plants that have been proven to be effective as larvacides, which include *Jacaranda mimosifolia*, *Tabebuia chrysantha*, *Tabebuia pallida* and *Tabebuia rosea*. These plants belong to the Bignoniaceae family.

One of the plants of the Bignoniaceae family is *Crescentia cujete* L. This plant can grow well in open areas exposed to direct sunlight and is found in both lowland and highland regions. *C. cujete* contains various secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, and saponins, which may contribute to its biological activity (Dasgupta & Mehta, 2023; Tan et al., 2025). However, the biological activity of *C. cujete* extracts is not necessarily limited to target organisms. Previous toxicity studies have demonstrated that responses to *C. cujete* extracts may vary depending on the plant part, extraction solvent, concentration, and test organism. For example, ethanolic extract of *C. cujete* fruit flesh showed cytotoxic activity against *Artemia salina*, with an LC_{50} of 529.386 ppm (Suzana & Handayanti, 2022). Similarly, methanolic extract of *C. cujete* leaves showed toxicity toward *A. salina*, with an LC_{50} of 642.877 ppm (Fatimah et al., 2022). These findings indicate that *C. cujete* extracts may affect non-target organisms and that their environmental safety cannot be assumed solely based on their plant origin. Therefore, further studies involving relevant non-target organisms and environmental assessments are required to determine the safety profile of *C. cujete* extracts. In the present study, the evaluation was limited to the larvicidal activity against *Ae. aegypti*, while toxicity toward non-target organisms and potential environmental effects were beyond the scope of the study.

Research on the larvicidal activity of plants belonging to the Bignoniaceae family has previously been conducted by Wulandari (2018). The study reported that ethanol extract of *C. cujete* fruit flesh exhibited larvicidal activity against *Ae. aegypti* instar II larvae, with LC₅₀ values of 0.40% after 8 h and 0.337% after 24 h of exposure (Wulandari, 2018). These findings provide evidence that the fruit flesh of *C. cujete* contains constituents with larvicidal potential. However, the larvicidal potential of other fruit parts, particularly the skin, remains less explored.

The selection of ethanol as the extraction solvent in the present study was based on evidence that extraction solvent can influence the phytochemical profile and the recovery of potentially bioactive constituents from *C. cujete*. Tan et al. (2025) reported differences in the phytochemical profiles of *C. cujete* fruit extracts obtained using different solvents, including dichloromethane, ethyl acetate, ethanol, methanol, and petroleum ether. These differences indicate that solvent polarity and extraction conditions can influence the chemical constituents recovered from the plant material. Therefore, ethanol was selected in the present study as an extraction solvent to obtain potentially bioactive constituents from both the fruit skin and flesh.

Previous studies have also demonstrated that the phytochemical characteristics of *C. cujete* may vary among different plant parts. Phytochemical profiling of the fruit flesh identified various metabolites, including phenolic acid derivatives, flavonoids, phenylethanoid derivatives, and iridoid glycosides (Rivera-Mondragón et al., 2020). Studies of other plant parts, such as the leaves, have also reported the presence of secondary metabolites including flavonoids, tannins, and saponins (Olaniyi et al., 2018). These findings suggest that differences in plant parts may influence the types and distribution of bioactive constituents present in *C. cujete*.

In particular, the fruit skin was included in the present study because its larvicidal potential has received less attention than that of the fruit flesh. Although the skin is an integral part of the fruit, it may possess a different phytochemical composition from the flesh and therefore may exhibit different biological activity. Recent phytochemical investigations of *C. cujete* fruit skin have reported the presence

of several classes of secondary metabolites, including flavonoids, phenolic compounds, alkaloids, terpenoids, steroids, and saponins, supporting the possibility that this plant part contains biologically active constituents (Purnamasari, 2025). Thus, investigating the fruit skin may provide additional information regarding its potential as a botanical larvicidal source.

Although other parts of *C. cujete*, such as seeds and leaves, may also contain bioactive compounds, their evaluation was beyond the scope of the present study. The study was specifically designed to compare the larvicidal activity of ethanol extracts obtained from the fruit skin and flesh against *Ae. aegypti*. In addition, the study included phytochemical screening to identify the major classes of secondary metabolites present in these two fruit parts. Comparing the skin and flesh may therefore provide additional information on differences in their phytochemical characteristics and larvicidal activity and may help identify which fruit part has greater potential as a source of botanical larvicidal compounds.

Based on the above reviews, this study aims to determine the effectiveness of ethanolic extract from skin and flesh of the *C. cujete* fruit as larvacides of *Ae. aegypti*, determine which concentration that has the most effect against larval mortality, and determine the content of active compounds that contained in the skin and flesh of the *C. cujete* fruit that can affect larval mortality

Methods

Preparation of Ethanol Extract from Skin and Flesh of The *C. cujete* Fruit

The materials used for the preparation of the extracts consisted of *Crescentia cujete* fruit skin and flesh. The skin and flesh were separated and dried in a dehydrator (Wirastar FDH10) at 45 °C for 2 days until a dry sample was obtained. The dried materials were then processed into simplicia.

For the extraction process, 100 g of each simplicia was subjected to maceration using 500 mL of 96% ethanol at room temperature for 2 × 24 h. After the first maceration, the mixture was filtered using filter cloth followed by filter paper to separate the

filtrate from the plant residue. The remaining residue was subsequently subjected to a second maceration using another 500 mL of fresh 96% ethanol under the same conditions. The two filtrates obtained from the successive maceration steps were combined and homogenized. Thus, the total volume of ethanol used was 1,000 mL per 100 g of simplicia, corresponding to an overall sample-to-solvent ratio of 1:10 (w/v). This successive maceration procedure was adapted from a previous study on *C. cujete* fruit extraction in which repeated maceration was performed and the resulting filtrates were combined before concentration (Suzana & Handayanti, 2023). The combined filtrate was subsequently concentrated using a shaking water bath (B-One SWB30) at 40°C for 2 days until a concentrated extract was obtained. The same extraction procedure was applied separately to the fruit skin and flesh.

Phytochemical Screening with Thin Layer Chromatography Test

Phytochemical screening using the Thin Layer Chromatography method is carried out by dripping the skin and flesh extracts that have been diluted with 96% ethanol onto a 10x4 cm silica gel F254 plate using a capillary tube.

Alkaloid

The motion phase used for the alkaloid test is methanol: ammonia (20:3) (Ismiyah et al., 2014). The test can be positive if there are orange, bluish purple, or brown spots under UV light 366 nm after spraying Dragendorf reagent (Widyaningsih et al., 2016).

Flavonoid

The motion phase used for the flavonoid test is toluene: ethyl acetate (12:8) (Fiisyatirodiyah et al., 2015) with FeCl₃ (Maulana, 2018). The test can be positive if there is a yellow, green, or blue spot under UV light 366 nm after spraying FeCl₃ 5% (Reveny, 2011).

Tannin

The motion phase used for the tannin test is n-Hexane: ethyl acetate (11:3) with FeCl₃ (Maulana, 2018). The test can be positive if there is a blackish brown spot under UV light 366 nm after spraying FeCl₃ 5% (Yuda et al., 2017).

Saponin

The motion phase used for the saponin test is chloroform: acetone (16:4) (Fiisyatirodiyah et al., 2015). The test can be positive if there is a purple or bluish green spot under UV light 366 nm after spraying Dragendorf reagent.

Triterpenoid

The motion phase used for the triterpenoid test is ethanol: chloroform (13.5:3). The test can be positive if there are orange, red, pink, yellow, green, and purple spots under UV light 366 nm after spraying Lieberman Bucard reagent (Anam, 2015).

Trapping and Hatching of *Ae. aegypti* eggs

The collection and rearing of *Aedes aegypti* were conducted under laboratory conditions following procedures adapted from the World Health Organization (WHO) guidelines for mosquito larval testing (WHO, 2005). *Ae. aegypti* eggs were collected using ovitraps placed in the environment of Universitas Negeri Malang. The ovitrap medium consisted of a mixture of ground water and boiled straw water at a ratio of 1:2. The ovitraps were maintained for 9 days and inspected periodically every 3 days. The collected eggs were transferred into containers containing ground water and maintained until hatching. The larvae were reared under laboratory conditions and fed fish food every 2 days at approximately 1–2 g. The water in the larval rearing containers was replaced with fresh ground water every 2 days. Third-instar larvae, approximately 3–4 days old and 4–5 mm in length, were selected for the larvicidal assay. The larvae were observed using a stereomicroscope (Olympus SZ61) before being used in the experiment. A total of 800 third-instar *Ae. aegypti* larvae were used in the larvicidal assay.

Effectiveness of Ethanol Extract from Skin and Flesh of The *C. cujete* Fruit as Larvacide.

The larvicidal activity of ethanol extracts from *Crescentia cujete* fruit skin and fruit flesh was evaluated using a bioassay procedure adapted from the WHO guidelines for laboratory testing of mosquito larvicides (WHO, 2005). Ethanol extracts of the fruit skin and flesh were tested at concentrations of 2,000,

3,000, and 4,000 ppm. A negative control containing no plant extract (0 ppm) and a positive control containing temephos were included.

For each treatment, 25 third-instar *Ae. aegypti* larvae were placed in a test cup containing 100 mL of the respective test solution. Each treatment was replicated four times. Larval mortality was recorded after 24, 48, 72, and 96 h of exposure. The temperature and pH of the test solutions were measured at each observation period. The concentrations, test-solution volume, and observation periods were determined based on the experimental design of the present study, while the general larvicidal bioassay procedure was adapted from the WHO guidelines (WHO, 2005).

Data Analysis

Data on chemical compounds obtained from phytochemical screening were analyzed descriptively. Mortality data of *Ae. aegypti* larvae obtained were tested for normality using Shapiro-Wilk and homogeneity using Levene test. After that, the data were tested Two-way Anova with a significance level of 5%. The next test used Tukey's test. Probit analysis to determine the Lethal Concentration (LC) using SPSS 25.0.

Results and Discussion

Contents of Secondary Metabolite Compounds in Ethanol Extracts from Skin and Flesh of The *C. cujete* Fruit.

The results of phytochemical screening using thin layer chromatography test are shown in Table 1. It can be seen that the positive results are in the identification of alkaloids, flavonoids, and triterpenoids. Thin layer chromatography results that show alkaloids are characterized by the formation of orange color both after and before spraying with dragendorf reagent on ethanol extract samples of fruit skin and bluish green color on ethanol extract samples of fruit flesh under UV light after being evaporated with methanol and ammonia. Positive results of flavonoids are characterized by the formation of blue color in the observation of ethanol extract of fruit flesh under UV light after being evaporated with toluene and ethyl acetate. Triterpenoid positive results are characterized by the formation of purple color in ethanol extract of fruit skin and green color in ethanol extract of fruit flesh with observation under UV light after being evaporated with ethanol and chloroform.

Table 1. Phytochemical Screening Results with Thin Layer Chromatography Test

Table IV. Phytochemical Screening Results with Thin Layer Chromatography Test									
Compounds	Extract	Resulting compound				Total Spot Color		Rf Value	Description
		After Elution		After Reagent Treatment		Before reagent treatment	After reagent treatment		
		Visual	UV 366 nm	Visual	UV 366 nm				
Alkaloid	Flesh	Brown	Bluish green	Brown	Bluish green	2	2	0,8125	+
	Fruit Skin	Brown	Orange	Brown	Orange	2	3	-	+
Flavonoid	Flesh	Brown	Blue	Brown	-	1	0	-	+
	Fruit Skin	Brown	Orange	Brown	-	0	0	-	-
Tannin	Flesh	Brown	Blue	Brown	-	0	0	0,8125	-
	Fruit Skin	Brown	Orange	-	-	0	0	0,125	-
Saponin	Flesh	Brown	Blue	Brown	Blue	0	0	0,25	-
	Fruit Skin	Brown	Orange	Brown	-	0	0	-	-
Triterpenoid	Flesh	Brown	Purple Green	Brown	Green	2	2	-	+
	Fruit Skin	Brown	Purple	Brown	Purple Green	2	2	0,8125	+

Notes: + = the tested samples contained the tested compounds; - = the tested sample does not contain the tested compound

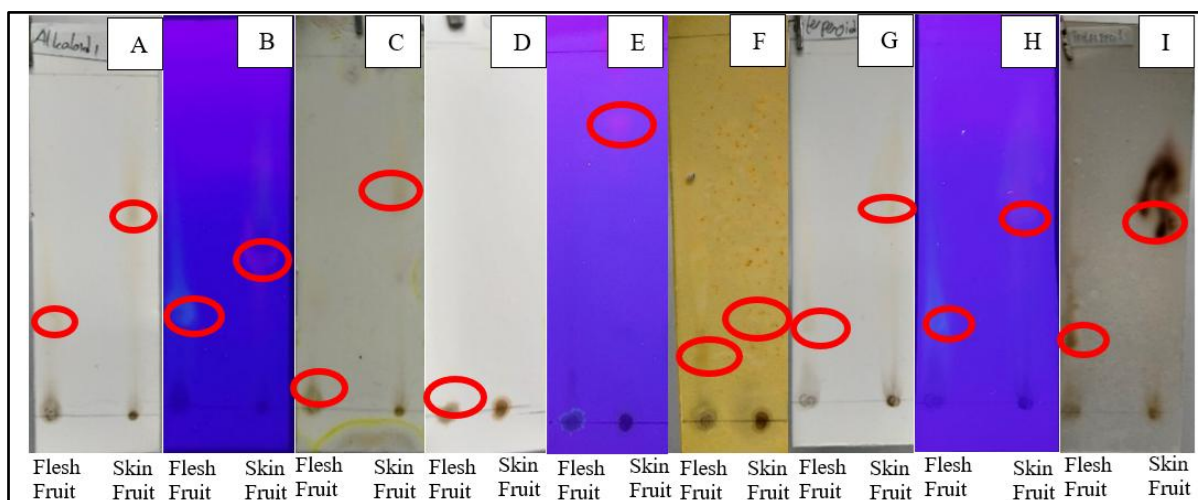


Figure 1. Phytochemical Screening Results Using thin layer chromatography test A) Alkaloid compounds after elution; B) Alkaloid compounds under UV light; C) Alkaloid compounds after being given Reagents; D) Flavonoid compounds after elution; E) Flavonoid compounds under UV light; F) Flavonoid compounds after being given Reagents; G) Triterpenoid compounds after elution; H) Triterpenoid compounds under UV light; I) Triterpenoid compounds after being given Reagents.

Table 1 shows the results of the calculation of the Rf value of each compound tested using the method. The Rf value for testing alkaloid compounds in ethanol extract of fruit skin is 0.8125 and ethanol extract of fruit flesh is 0.125 with methanol and ammonia eluents using dragendorf spotting. The Rf value for testing flavonoid compounds in ethanol extracts of fruit peel cannot be calculated because there are no color spots that appear during testing, while the Rf value of flavonoid compounds in ethanol extracts of fruit flesh is 0.25 with eluents used are toluene and ethyl acetate using FeCl₃ spots. The Rf value for testing tannin compounds in ethanol extracts of fruit skin cannot be calculated because there are no color spots that appear during testing and while the Rf value of tannin compounds in ethanol extracts of fruit flesh is 0.0625 with eluent n-Hexane and ethyl acetate using FeCl₃ spotting. The Rf value for testing saponin compounds in ethanol extract of fruit peel is 0.1875 and ethanol extract of fruit flesh is 0.25 and testing on ethanol extract of fruit peel cannot be calculated because there are no color spots that appear when testing with chloroform and acetone using Dragendorf spotting. The Rf value for testing triterpenoid compounds of fruit peel ethanol extract is 0.8125 and fruit flesh ethanol extract is 0.8125 with chloroform and acetone eluents using Lieberman Burchard reagent.

Figure 1 shows that the ethanol extract of fruit skin and ethanol extract of fruit flesh contains alkaloid compounds seen from the color spots that appear before and after UV light showing orange and bluish green colors with a total of 2 spots appearing on the fruit skin before the reagent and 2 after the reagent, while the ethanol extract of fruit skin shows a total of 2 spots before the reagent and 3 after the reagent. Ethanol extract of fruit flesh contains flavonoid compounds seen from the color spots that appear before and after UV light showing blue color with a total spot that appears is 1 before the reagent and 0 after the reagent. Ethanol extract of fruit skin and ethanol extract of fruit flesh contains triterpenoid compounds seen from the color spots that appear before and after being given light showing purple and green colors with a total of spots that appear on the fruit skin 2 before the reagent and 2 after the reagent, while the fruit skin shows a total of 2 spots before the reagent and 2 after the reagent.

The results of phytochemical screening using thin layer chromatography test showed that alkaloid compounds have the best visualization results among other compounds with eluents used are methanol and ammonia, where both solutions have a high level of polarity (Adnan, 1997). The level of solvent polarity affects the ethanol extract from skin and flesh of The *C. cujete* fruit, so that in testing tannin and saponin compounds do not show visual color spots on silica plates. This shows

that the solution used is not suitable for testing these two compounds, because n-hexane, acetone and ethyl acetate have a low level of polarity (Adnan, 1997). Polar secondary metabolites such as flavonoids and tannins penetrate the polar peptidoglycan layer more easily than the non-polar lipid layer. In addition, the cell walls that are most easily denatured are cell walls composed of polysaccharides compared to cell walls composed of phospholipids (Gultom et al., 2021).

Thin layer chromatography test results provide results that the ethanol extract of *C. cujete* fruit contains alkaloid, flavonoid, and triterpenoid compounds. However, tests conducted by Ejelonu et al. (2011) stated that the presence of saponin compounds was identified in *C. cujete* fruit. This shows that there are factors that can affect the non-detection of secondary metabolite compounds. The concentration of the solution tested is thought to have an effect because the extract solution used is not too concentrated so that it can affect the test results. According to (Kagan & Flythe, 2014) the dilution of the extract can be done in a ratio of 1: 1, namely 1 drop of extract for 1 mL of solvent. The solvent used to dissolve the extract is considered too much so that the solution obtained becomes not concentrated so that the tannin and saponin compounds of *C. cujete* fruit ethanol extract cannot be detected. Differences in environmental conditions where plants grow are also one of the causes of differences in secondary metabolite content (Sampaio et al., 2016).

Effect of Ethanol Extract from Skin and Flesh of The *C. cujete* Fruit as Larvacide on Mortality of *Ae. aegypti* Larvae.

Based on the results of research testing data of the effectiveness of Ethanol Extract from Skin and Flesh of The *C. cujete* Fruit on *Ae. aegypti* mosquito larvae that experience death with various variations of different concentrations during exposure times of 24 hours, 48 hours, 72 hours and 96 hours are presented in Table 2.

Table 2 shows that out of 100 *Ae. aegypti* larvae after 24 hours of treatment with various concentrations, there was the highest percentage of mortality of *Ae. aegypti* larvae of 29% at a concentration of 4000 ppm ethanol fruit skin extract. In the negative control, using aquades, the number of larvae that died was 0% and in the positive control the number of larvae that died was 100%. In the observation of *Ae. aegypti* larvae after 48 hours of treatment with various concentrations, there was the highest percentage of death of *Ae. aegypti* larvae of 60% at a concentration of 4000 ppm ethanol extract of fruit flesh. In the observation of *Ae. aegypti* larvae after 72 hours treated with various concentrations, there was the highest percentage of death of *Ae. aegypti* larvae of 68% at the concentration of ethanol extract of fruit skin 4000 ppm. In the observation of *Ae. aegypti* larvae after 96 hours of treatment with various concentrations, there was the highest percentage of death of *Ae. aegypti* larvae of 73% at a concentration of 4000 ppm fruit skin ethanol extract. In quantity, each treatment group increased the number of larval deaths along with the increase in treatment concentration, this is in accordance with research conducted by Rahayu et al. (2021) which states that the higher the concentration of the extract and the observation time, the higher the mortality of the *Ae. aegypti* larvae, so the higher the concentration of *C. cujete* fruit, there is also a higher mortality of *Ae. aegypti* larvae.

Table 2. Frequency distribution of mortality of *Ae. aegypti* mosquito larvae by concentration variation

Treatment	concentration (ppm)	Percentage (%) Mortality Hour-			
		24	48	72	96
Control	+	100	100 ^c	100 ^c	100 ^c
	-	0	0 ^a	0 ^a	0 ^a
Skin Fruit	2000	10	32 ^b	43 ^b	48 ^b
	3000	13	37 ^c	49 ^c	60 ^c
	4000	29	55 ^d	68 ^d	73 ^d
Flesh Fruit	2000	10	24 ^b	35 ^b	48 ^b
	3000	19	40 ^c	52 ^c	59 ^c
	4000	25	60 ^d	67 ^d	72 ^d

Notes: (+) =using temephos (-) =using aquades

The results of the Two-way Anova test showed that there were no differences in the larvicidal treatment of ethanol extract of fruit peel and ethanol extract of fruit flesh on the mortality of *Ae. aegypti* larvae and showed there were differences in larvicidal concentrations of ethanol extract from Skin and flesh of the *C. cujete* fruit on the mortality of *Ae. aegypti* larvae. For the results of the Two-way Anova test for the combination of treatments and concentrations, there were no differences between treatments and larvicidal concentrations of ethanol extract from Skin and flesh of the *C. cujete* fruit on the mortality of *Ae. aegypti* larvae. Then, further post hoc test analysis was conducted with Tukey HSD test to determine significant differences between concentrations of 0 ppm, 2000 ppm, 3000 ppm, 4000 ppm with positive control (Temephos) at various hours of observation. Table 3 shows that there is a significant difference between the concentration and the positive control.

The test results showed that the ethanol extract from Skin and flesh of the *C. cujete* fruit gave an effect in the form of death of *Ae. aegypti* instar III larvae at all concentrations of ethanol extract from Skin and flesh of the *C. cujete* fruit with an average number of deaths that increased every hour. The higher the concentration used, the higher the number of larval deaths.

The mortality of *Ae. aegypti* larvae observed following exposure to the ethanol extracts of *C. cujete* skin and flesh may be associated with the secondary metabolites detected during the phytochemical screening. In the present study, alkaloids and triterpenoids were detected in both skin and flesh extracts, whereas flavonoids were detected only in the flesh extract. Previous studies have reported

that plant-derived alkaloids, flavonoids, and terpenoids can exhibit larvicidal activity against *Ae. aegypti* through different mechanisms, including interference with the nervous system, disruption of respiration, alteration of digestive processes, and effects on other physiological pathways (Ganesan et al., 2023; Teles et al., 2024). In particular terpenoids have been reported to affect mosquito larvae through mechanisms involving acetylcholinesterase inhibition, oxidative stress, mitochondrial dysfunction, and disruption of larval morphology (Teles et al., 2024). Alkaloids have also been identified among plant secondary metabolites with demonstrated larvicidal activity against *Ae. aegypti* (Ganesan et al., 2023; Wuillda et al., 2019). Therefore, the presence of alkaloids and triterpenoids in both extracts, together with flavonoids in the flesh extract, may be associated with the larvicidal activity observed in the present study. However, because the phytochemical analysis conducted in this study was qualitative and did not quantify individual compounds or establish their direct mechanisms of action, the contribution of each phytochemical group to larval mortality cannot be conclusively determined.

Biological activity such as toxicity is influenced by the content of compounds in the extract (Altemimi et al., 2017), so the cause of larval death is influenced by the content of secondary metabolite compounds present in *C. cujete* extract. Differences in solvents for extraction and the location of growing plants affect the chemical compounds that can be extracted (Widyawati et al., 2014). Efforts are needed to obtain effective solvents to extract phytochemical compounds that act as larvicides (To'bungan & Jati, 2022).

Table 3. Tukey 5% test results based on the percentage of mortality of *Ae. aegypti* larvae at various concentrations

No	Concentration (ppm)	Percentage (%) mortality of <i>Ae. aegypti</i> larvae based on observation time $\pm$ SE		
		48 Hour	72 Hour	96 Hour
1.	0	0,00% ^a + 3,719	0,00% ^a + 1,732	0,00% ^a + 2,677
2.	2000	28,00% ^b + 3,221	39,00% ^b + 1,732	48,00% ^b + 2,677
3.	3000	38,50% ^c + 3,221	50,50% ^c + 1,500	59,50% ^c + 2,318
4.	4000	57,50% ^d + 3,221	67,50% ^d + 1,500	72,50% ^d + 2,318
5.	Temephos	100,00% ^e + 3,719	100,00% ^e + 1,500	100,00% ^e + 2,318

Table 4. Results of Probit Analysis of LC Values after application of extracts on Mortality of *Ae. aegypti* Larvae.

Ekstrak	Lethal Concentration (%)	Observation Time (hour)/Estimate (ppm)		
		48	72	96
Skin Fruit	LC ₅₀	4556.794	4032.161	3415.007
Flesh Fruit	LC ₅₀	3996.011	3596.637	3112.767

Effective Concentration of Ethanol Extracts from Skin and Flesh of The *C. cujete* Fruit

The results of probit analysis with a 5% significance level were carried out to obtain the LC₅₀ value. Probit LC₅₀ analysis to determine the most effective concentration in causing death in *Ae. aegypti* larvae. The results of the analysis of each sample of ethanol extract of fruit skin and ethanol extract of fruit flesh can be seen in Table 4.

The results of Probit Analysis in table 4 show the LC₅₀ value at 48 hours of observation obtained with the concentration of ethanol extract of fruit skin 4556.794 ppm and for the concentration of ethanol extract of fruit flesh is 3996.011 ppm. LC₅₀ value at 72 hours observation was obtained with the concentration of ethanol extract of fruit skin 4032.161 ppm and for the concentration of ethanol extract of fruit flesh is 3596.637 ppm. LC₅₀ value at 96 hours observation was obtained with the concentration of ethanol extract of fruit skin 3415.007 ppm and for the concentration of ethanol extract of fruit flesh is 3112.767 ppm. The data shows that the lower the LC value of a substance, the substance has a higher activity to kill test animals. This is in accordance with research conducted by Jelita et al. (2020) which states that the smaller the LC₅₀ value of a sample, the higher its bioactivity. Based on this, the ethanol extract of the fruit flesh is considered to have high effectiveness as a larvicide because it has a low LC.

Conclusion

The results of this study indicate that ethanol extracts of the skin and flesh of *C. cujete* fruit contain secondary metabolites, including alkaloids, flavonoids, and triterpenoids, as detected by phytochemical screening. Both extracts demonstrated larvicidal activity against *Ae. aegypti*, with the highest mortality observed at a concentration of 4000 ppm after 96 h of

exposure. At this concentration, the ethanol extract of the skin caused 73% mortality, while the ethanol extract of the flesh caused 72% mortality. The LC₅₀ values at 96 h were 3415.007 ppm for the skin extract and 3112.767 ppm for the flesh extract. These findings suggest that ethanol extracts of *C. cujete* skin and flesh have potential as plant based alternative larvicidal agents against *Ae. aegypti*. However, further studies are required to evaluate their efficacy under field conditions, determine their effects on non-target organisms, and assess their safety, stability, and appropriate formulation before they can be considered for practical application as alternative larvicides.

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