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Silver Nanoparticle of *Acalypha indica* Linn. Leaf As Bio-larvicide against *Anopheles* sp. Larvae

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Abstract

BACKGROUND: *Acalypha indica* Linn. has been used as traditional medicine, it contains flavonoids, alkaloids, tannins, saponins, steroids, triterpenoids, and essential oils.

AIM: This study aimed to determine the bio-larvicide effects of *A. indica* Linn. leaf stew and the silver nanoparticles against *Anopheles* sp. larvae.

METHODS: The fresh leaves of *A. indica* Linn. extracted using distilled water at 100°C for 30 min. The silver nanoparticles were made by mixing a solution of silver nitrate with the stew, which acts as a reducing agent. The resulting silver nanoparticles were characterized by particle size analyzer and UV-vis spectrophotometer. The bio-larvicide effects against *Anopheles* sp. larvae performed using a completely randomized design. There were eight groups consisted of ten larvae and three replications. Treatment groups of stew and silver nanoparticle for concentrations 0.05%, 0.5%, and 5%, respectively. The negative control group was distilled water and the positive control group was the 0.01% abate solution. Assessment of larvicide activity was carried out every hour for 6 h and continued if there were larvae that live up to 24 h. The LC_{50} value was calculated based on Probit analysis.

RESULTS: The results showed that the *A. indica* Linn. leaf stew can be made into silver nanoparticles preparations, optimal results were obtained from a mixture of 1% stew and 3 mM $AgNO_3$. The result of bio-larvicides effect test against *Anopheles* sp. larvae showed that the LC_{50} value of the *A. indica* Linn. leaf stew was 727,3 ppm and the LC_{50} value of silver nanoparticles was 3.366 ppm.

CONCLUSION: It can be concluded that *A. indica* Linn. is a promising larvicidal plant and can be made into silver nanoparticle preparations.

Introduction

Malaria disease is caused by the *Plasmodium* parasite, which is spread by female *Anopheles* mosquitoes. According to the WHO report, globally, there were 229 million cases in 2019 with a mortality rate 10 in 100.000 population at risk [1]. Handling malaria can be done with prevention and treatment. Prevention can be done using insect repellent lotion, removing breeding places and standing water around the house, and killing mosquito larvae by fogging or using larvicides [2]. Chemical substances and natural materials can be used as larvicides. Research on the larvicidal effect has been carried out on several plants for example *Acalypha indica* Linn. [3], [4], Solanum xanthocarpum, Euphorbia tirucalli, Momordica charantia, Eucalyptus globules, Citrullus colocynthis, A. indica, Annona squamosa, and Solanum nigrum [5].

A. indica Linn. is a type of weed wild plants that are often found on the roadside, untreated grass fields even as a nuisance on agricultural land [6]. According to Hayati *et al*., the components contained in this plant are β-sitosterol and daucosterol, saponins, tannins, flavonoids, and essential oils [7]. The community has been used this plant as traditional medicine and the results of the researches prove its efficacy, among others, as antidiabetic, anti-inflammatory, antioxidant, anti-poison, anticancer, hepatoprotective, antibacterial, wound healing, anthelmintic, repellent, and larvicide [3], [8], [9], [10]. Research conducted by Pratiwi *et al*. (2015) shows that the ethyl acetate extract of *A. indica* Linn. has a larvicidal effect on *Aedes aegypti* mosquito larvae with an LC_{50} of 72.444 ppm [10].

Pharmacological effects possessed by natural substances are produced by the content of secondary metabolites. Several methods can be used to find the content of natural ingredients, generally distinguished by the hot method and cold method. The choice of method is based on the physical and chemical properties of the secondary metabolites to be explored. The decoction is one of the simple extraction methods using water as a

solvent; the material to be extracted must be resistant to heating [12].

Several studies that use natural ingredients try to compare the pharmacological effects produced between plant extracts and plant nanoparticle preparations. The results obtained indicate that an increase in the effect obtained from the preparation of nanoparticles, due to the small particle size of the drug which allows the drug to be absorbed and penetrate quickly into cells and provide the therapeutic effect. Silver nanoparticles are one of the nanoparticle preparations that have been developed. Nano-sized silver metal particles have antimicrobial properties but are environmentally friendly and safe for humans [13], [14]. The green synthesis method uses plant extracts that act as reducing agents which react with metal ions to produce silver nanoparticles. Plant parts can be used in the form of leaves, fruit, or seeds [14]. This study aimed to make the *A. indica* Linn. leaf stew and silver nanoparticle preparations, and determine the bio-larvicide effect against *Anopheles* sp. larvae.

Materials and Methods

Materials

A. indica Linn. leaves were collected in June from Malalayang district at Manado City. $AgNO_3$ purchased from Merck Company. Polyvinyl alcohol (PVA) purchased from Sigma-Aldrich. Abate purchased from BASF Indonesia. Test animals were *Anopheles* sp. third instar larvae purchased from Department of Environmental Health, Politeknik Kesehatan Kementerian Kesehatan Manado, Indonesia.

Methods

Stew and silver nanoparticles preparation

1. Stew preparation: *A. indica* Linn. leaves taken in the morning and washed with running water, and then the leaves were air-dried. Furthermore, the leaves were coarsely powdered using a grinder. The leaves powder weighed 20 g and placed in Erlenmeyer containing 200 mL of distilled water, then simmer for 30 min. Then the solution was cooled to room temperature then filtered with Whatman filter paper no. 1, and the stew was stored in a cold storage cabinet at 4°C. For the nanoparticle preparation, the stew was diluted with aquadest to obtain a concentration of 0.5, 1, and 5% [15].
2. Silver nanoparticle preparation: Optimization of the nanoparticles preparation carried out by adding 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, and 2000 μL of 1% *A. indica* Linn. leaf stew to 1 mL of 1 mM $AgNO_3$ solution. Also made preparations using variations in the concentration of $AgNO_3$ 1–5 mM added with the 0.5 and 5% *A. indica* Linn. leaf stew. As a stabilizer 0.5% PVA solution was added for every 2 mL $AgNO_3$. The mixture was allowed to stand in a dark container at cool temperatures for 24 h. The formation of silver nanoparticles was characterized by a change in color of the solution from colorless to slightly turbid brown with wavelengths of 430–480 nm and particle size of 100 nm ≥[15]. The silver nanoparticle preparation then processed using a freeze dryer.
3. Characterization of silver nanoparticle:

a. UV-Vis spectrophotometer
UV-Vis spectrophotometer was standardized using blank. The extract solution was put in a cuvette and then measured at a wavelength of 350–500 nm [14].

b. Particle size and morphology analysis
The particle size of the extract preparation measured using a Particle Size Analyzer (PSA) [14], [16]. The formulation that has the smallest particle size with the appropriate wavelength was then used in the bio-larvicide test.

Secondary metabolites test

The test of the secondary metabolites content in the stew was carried out qualitatively using chemical reagents to determine alkaloid, tannin, terpenoid, steroid, flavonoid, and saponin.

Bio-larvicide test

A total of 10 *Anopheles* sp. third instar larvae transferred from the container into a vial containing the *A. indica* Linn. stew with a concentration of 0.05%, 0.5%, and 5%, control (+) by giving an abate concentration of 0.01%, and control (−) by giving distilled water. Mosquito larvae activity of *Anopheles* sp. was observed for 24 h. Observation of dead larvae was carried out at the hours 1, 2, 3, 4, 5, and 6. Observation of the flow of life was continued if there were larvae that lived until 24 h after treatment. Calculation of time begins after the insertion of larvae into glass beakers. Observation of the life cycle, that is, the test larvae were given extracts able to survive for a certain period of time but could not reach the next stage. Larvae are considered dead if there are no more signs of life, for example, do not move anymore even though stimulated by the movement of water and touched with a stick [11].

The data obtained were made in the form of tables and graphs and LC_{50} values were calculated

based on Probit analysis.

Results

Stew and silver nanoparticles preparation

From 110 samples with varying concentrations of *A. indica* Linn. stew and $AgNO_3$, there were six samples that met the criteria of wavelength 430–480 nm and particle size on the nanometer scale, as shown in Table 1 and Figure 1.

Table 1: Optimization results of silver nanoparticles of *Acalypha indica* Linn. leaves

Sample code	Mix of	λ (nm)	Particle size (nm)			
			D1	D2	D3	Mean
B1	AILS 1% - $AgNO_3$ 3 mM	460, 449, 441	106,7	100,6	104,9	104,1
B2	AILS 1% - $AgNO_3$ 3 mM	446	88,2	96,8	108,2	97,7
B3	AILS 1% - $AgNO_3$ 3 mM	451	91,8	82,5	91,8	88,7
B4	AILS 1% - $AgNO_3$ 3 mM	442	100,8	101,5	99,6	100,6
F1	AILS 0,5% - $AgNO_3$ 5 mM	431	130,0	128,1	129,6	129,2
G3	AILS 1% - $AgNO_3$ 5 mM	468	93,1	92,2	91,8	92,4

AILS: *Acalypha indica* Linn. leaves stew

Based on these results, those who met the criteria as silver nanoparticles *A. indica* Linn. leaves was B3 samples because they have the smallest particle size.

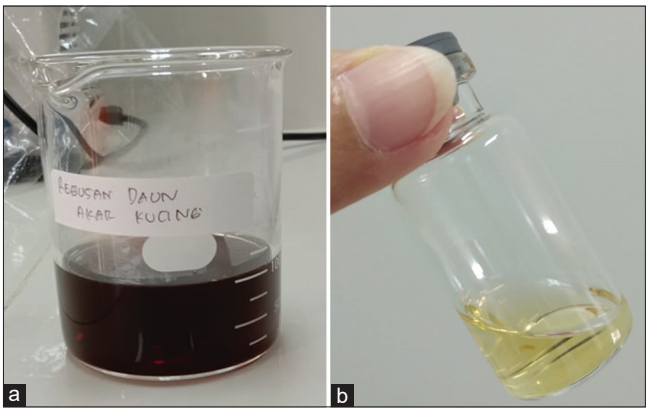

Figure 1: Acalypha indica Linn. leaves stew (a), silver nanoparticles of A. indica Linn. leaves stew (b)

Secondary metabolites test

The test conducted using chemical reagents to determine alkaloid, tannin, terpenoid, steroid, flavonoid, and saponin in the stew. The result showed that all the secondary metabolites tested were contained in the stew (Table 2). The content of these secondary metabolites produce pharmacological effects.

Table 2: Secondary metabolites in the stew of Acalypha indica Linn. leaves

Secondary metabolites test	Result
Alkaloid	+
Saponin	+
Tannin	+
Flavonoid	+
Steroid	+
Terpenoid	+

+: Contains the compound, −: Non contains the compound.

Bio-larvicide test of stew and silver nanoparticles of A. indica Linn. leaf stew

The results of the bio-larvicides test of *A. indica* Linn. leaf stew and silver nanoparticles of *A. indica* Linn. leaf stew against the larvae of *Anopheles sp*. Are presented in Table 3.

Table 3: Bio-larvicides effects of stew and silver nanoparticles of *Acalypha indica* Linn. leaf stew in the larvae of *Anopheles* sp.

Sample	Mortality percentage of *Anopheles* sp. mosquito larvae			
	N1	N2	N3	Mean
Positive control	100	100	100	100
Negative control	0	0	0	0
Stew 0,05%	60	50	50	53,33
Stew 0,5%	80	80	70	73,33
Stew 5%	100	100	100	100
Silver Nanoparticle 0.05%	90	90	90	90
Silver Nanoparticle 0.5%	100	100	100	100
Silver Nanoparticle 5%	100	100	100	100

Based on these data, the LC_{50} value is calculated based on the Probit analysis as follows in Table 4.

Table 4: Probit analysis data for LC_{50} calculations

Sample	Concentration (%)	Log	% Mortality	Probit value
Stew	0.05	−1.30103	53.3	5.08
Stew	0.5	−0.30103	73.3	5.61
Stew	5	0.69897	100	8.09
Nanoparticle	0.05	−1.30103	90	6.48
Nanoparticle	0.5	−0.30103	100	8.09
Nanoparticle	5	0.69897	100	8.09

From the regression equation, the LC_{50} value for *A. indica* Linn. leaf stew is 0.07273%, if the value is converted to the weight of the *A. indica* Linn. leaf it will be equivalent to 10 fresh leaves/100 mL. While, the LC_{50} value for nanoparticles of *A. indica* Linn. stew leaf is 0.0003366%.

Discussion

Samples of *A. indica* Linn. leaves used in dry form. The drying process by air-dried to prevent damage to the content in the leaves due to the sunlight. Stew is one of the simple methods of extracting heat using water as a solvent [11]. The choice of method and solvent is based on the physical and chemical properties of the secondary metabolites to be explored. Polar materials such as flavonoids, saponins, and tannins are extracted with polar solvents such as water and n-butanol [17]. Most flavonoid content is found in the leaves of this plant [18]. The stew was obtained in the form of dark brown liquid with a specific aroma like *A. indica* Linn.

To determine the right formulation of silver nanoparticles, the optimization of the preparations

was done using various comparisons of several concentrations of stew and $AgNO_3$ solution. The formation of silver nanoparticles is characterized by a brownish yellow color as seen in Figure 1. To ensure, the measurement of the wavelength is compared with the stew of the *A. indica* Linn. leaf. Silver nanoparticles have a wavelength of 430–480 nm, while the stew has a wavelength of 300–380 nm. The particle diameter measurement is done using the PSA. The sample chosen for testing the effects of bio-larvicides was those that had the smallest particle size. The small particle size allows the drug to absorb and penetrate rapidly into cells and provide a therapeutic effect.

The secondary metabolite test conducted using chemical reagents. The result showed that alkaloid, tannin, terpenoid, steroid, flavonoid, and saponin found in the stew. The previous study results showed that this plant contains β-sitosterol and daucosterol, saponins, tannins, flavonoids, and essential oils [7]. This plant has been used in traditional medicine as antidiabetic, anti-inflammatory, antioxidant, anticancer, hepatoprotective, antibacterial, wound healing, anthelmintic, and larvicide [8], [9].

The bio-larvicide test uses *Anopheles* sp. third instar larvae and observations are carried out every hour for 6 h and if there are still mosquitoes that do not die then the observations are carried out for up to 24 h to observe the life path. The third instar larvae were chosen for the test because the size is larger than the first and the second instar larvae so that the observation becomes easier. Besides that, the third instar larvae are more resistant to current mechanical factors [10]. The results showed that the greater the stew concentration, the greater the percentage of mosquito larvae mortality and the larvae that did not die while the experience period would not grow to the next phase. Komalamisra *et al*. [19] considered larvicidal products exerting LC_{50} <50 mg/L (active), 50 mg/L <LC_{50} <100 mg/L (moderately active), 100 mg/L <LC_{50} <750 mg/L (effective), and LC_{50} >750 mg/L (inactive). Kiran *et al*. [20] considered compounds with LC_{50} <100 mg/L as exhibiting a significant larvicidal effect.

Phytochemicals such as alkaloids, phenols, and triterpenoids alone or in combination, contribute to acute toxicity toward various arthropod species [21]. The death of mosquito larvae in this research is presumed due to the secondary metabolites content in *A. indica* Linn. leaves, that is, alkaloid, triterpenoid, flavonoid, tannin, saponin, and steroid. Alkaloid compounds work by disrupting the system nerves (neuromuscular toxic), inhibiting the feeding power of the larva, and acts as a stomach poison. The mechanism of action of the alkaloids, namely by inhibiting the action of the enzyme functioning acetylcholinesterase hydrolyzes acetylcholine. In a state of acetylcholine normally conducts function nerve implants, after which it is immediately experienced hydrolysis with the help of acetylcholinesterase into choline and acetic acid. With the help of this acetylcholinesterase enzyme occurs build-up of acetylcholine to be causes disturbance and damage in the nervous system [22]. Alkaloids also act as an antifeedant and stomach poisoning [23]. Flavonoids act as an insecticide by attacking the nerves in some vital organs of insects, thus arising a weakening of the nerves such as the inhalation resulting in the larvae unable to breathe and finally died. Flavonoids work as a respiratory inhibitor and are considered to disturb the energy metabolism in the mitochondria by inhibiting the conveying system electrons [24]. Triterpenoids are the active components of the limonoid compounds that are toxic used as an insecticide as potential as an antifeedant against insects, growth regulators, and substances toxic to rice lice, larvicides, antimicrobial, insect repellent, and reproductive inhibitor [25]. Steroids have a chemical structure similar to triterpenoids. Steroids are hormone growth that affects the molting of the larvae. Steroids will cause chitin cell walls in the body larvae to thicken; resulting in larval growth will be disturbed and cause death in larvae [26]. Saponins cause a decrease in the activity of digestive enzymes and the absorption of food in insects [27]. In addition, saponins also damage the cuticle membrane of larvae and cause the death of larvae [28]. The tannin compounds contained to act as digestive toxins and interfere with water absorption in larvae so that they can cause death [27], [29].

The LC_{50} value shows that nanoparticle preparation has much stronger bio-larvicide effects than the stew. This is possible because of its small size making it easier to penetrate cells, as well as the presence of nano-sized silver metal content that has antimicrobial properties but is environmentally friendly and safe for humans [13]. The results obtained in this study indicate the potential for the development of stew and silver nanoparticles of the *A. indica* Linn. leaves to be used as bio-larvicides.

Conclusions

A. indica Linn. leaves can be extracted with a simple method of stew and the results of the stew can be made into silver nanoparticle preparations. *A. indica* Linn. is a promising larvicidal plant in which the LC_{50} value of the leaf stew and its silver nanoparticles were 727.3 ppm and 3.366 ppm, respectively. It is recommended to conduct similar research on mosquito larvae that cause other diseases such as *A. aegypti* and Culex sp. as well as conducting further research on the stability of silver nanoparticle preparations.

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Credit Author Statement

YB: Conceptualization, Methodology, Investigation, Writing-Original draft, Writing- Reviewing and Editing; OS: Visualization, Validation; EB: Formal analysis, Supervision; SS: Resources, Methodology; RW: Resources, Conceptualization; SU: Project administration, Data curation; ZS: Writing- Reviewing and Editing, Data curation.

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