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# The Potency of Anting Anting (*Acalypha indica* L.) Leaf Extract as An Acaricide on *Boophilus microplus* in Larvae and Adult Stages *In Vitro*

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**Abstract**

The purpose of this study was to determine the potency of anting-anting leaf extract (*Acalypha indica* L.) as an acaricide against larval and adult *Boophilus microplus* in vitro by observing the mortality rate of *B. microplus*. The present study design was a completely randomized design. This study used five treatments, including: Tween 80 1% and aquadest (K-), Neguvon (K+), anting-anting leaf extract with a concentration of 3.125% (P1), 6.25% (P2), and 12.5% (P3 ). Each treatment used 5 repetitions and each repetition used 6 *B. microplus* larval stages and 5 *B. microplus* adults. Observations were made for 5 hours for the larval stage of *B. microplus* and 24 hours for the adult stage of *B. microplus*. *Boophilus microplus* is declared dead if there is no movement at all when touched with a needle. The data obtained were analyzed using factorial ANOVA and continued with Duncan's multiple distance test. The results showed that the 12.5% anting anting leaf extract treatment showed no significant difference with the treatment using Neguvon. ($p>0.05$) in larval stage *B. microplus*, but in adult *B. microplus* showed a significant difference with Neguvon ($p<0.05$). The results of the statistical test can be concluded that the extract of anting-anting leaf (*A. indica* L.) has the potential as an acaricide of *B. microplus* larval stage in vitro. The higher the concentration of anting anting leaf extract, the higher the acaricide activity.

**Keywords:** *Acalypha indica*, *Boophilus microplus*, acaricide, leaf extract, in vitro, Adult stage.

## Introduction

Boophilus microplus is an ectoparasite that often causes problems for cattle breeders in Indonesia. *Boophilus microplus* infestation causes various major losses such as: decreased body weight, decreased milk production, impaired comfort in livestock, and skin damage (Avinash *et al*., 2017). *Boophilus microplus* also acts as a vector for blood protozoan diseases such as: Anaplasmosis, Babesiosis, and Theileriosis (Soulsby, 1982). Health problems, especially those related to B. microplus, have not been completely addressed. Losses due to *B. microplus* infestation continue to increase from year to year (Adenubi *et al*., 2016). One of the methods used by cattle breeders to control cow ticks is to use chemical acaricides. Some examples of chemical acaricides include: Coumaphos (Asuntol), Propoksur, Cypermethrin and Pyrethroids (Husna, 2014). The use of chemical acaricides in livestock is not necessarily safe, given the presence of harmful chemicals in acaricides. The impacts due to the use of chemical acaricides include: causing poisoning or even death in livestock, killing non-target organisms, and accumulation of acaricide residues in either meat or milk (Kumar *et al*., 2016). In addition, the uncontrolled use of chemical acaricides can lead to the emergence of resistant tick lines (Abbas *et al*., 2014). In connection with various problems and losses caused by *B. microplus* infestation, it is necessary to alternative acaricides as an effort to control cow ticks. One alternative that can be done is to use herbal plants that have the potential to control the population of cow ticks. The use of herbal acaricides has the advantage of being easy to obtain in nature and easy to decompose so that not to pollute the environment (Rohimatun and Wiratno, 2015). Plants that can be used as an alternative to acaricides are anting anting plants (*Acalypha indica* L.). The phytochemical content of anting anting leaves includes alkaloids, tannins, steroids, saponins, flavonoids, glycosides, acalyphin and phenol (Mohan *et al*., 2012). Previous study has shown that anting

anting leaf extract can be used as an in vitro anti-scabies (Astuti, 2019) and larvicide for Anopheles stephensi mosquitoes (Govindarajan *et al*., 2008). Based on the description above, it is necessary to conduct research to determine the acaricide potential of anting anting leaf extract against *B. microplus* with various concentrations.

## Materials and Methods

The research design used in this study was a completely randomized design (CRD). Each treatment used five repetitions and each replication used 6 larval stage *B.microplus* and 5 adult stage *B.microplus.* The treatments consisted of: Tween 80 1% and aquadest (K-), Neguvon (K+), ethanol extract of anting anting leaves with a concentration of 3.125% (P1), 6.25% (P2), and 12.5% (P3) . Observations on the larval stage of *B. microplus* were carried out for 5 hours, while for the adult stage of *B. microplus* it was carried out for 24 hours. *B. microplus* larvae are declared dead if there is no reaction to movement when touched with a needle and generally the legs of the dead larvae look like they are rolled up (Wardhana *et al*, 2005). Adult *B. microplus* is declared dead if when touched there is no response to movement and the tick's cuticle looks dark (Fernández-Salas, 2011). The process of rearing adult female *B. microplus* that was full of blood was carried out in Pandantoyo Village, Ngancar District, Kediri Regency. *Boophilus microplus* adult females that have been filled with blood are placed in a container that has been lined with filter paper. During the rearing process the humidity is maintained by spraying water on the filter paper. The container is closed using a folded gauze. The container is placed in a box. The optimal environmental temperature and humidity for incubation of *B. microplus* eggs is 20°C-31°C with 70-90% humidity (Hitchcock, 1955). The larvae of *B. microplus* used in this study were 7 days old (Sindhu *et al*., 2012).

## Data Analysis

The data obtained were analyzed by ANOVA ($\alpha = 0.05$), if there was a difference in the treatment data, it was continued with Duncan's double spaced test. The values of Lethal Concentration 50 (LC50) and Lethal Concentration 90 (LC90) were analyzed using probit analysis test. Statistical analysis was carried out using the SPSS for windows 23 program.

## Results and Discussion

**Table 1**. Mean and Standard Deviation of Mortality of *B.microplus* Larvae Stadium Based on Interaction of Treatment and Time

| Hour / Treatment | 0-1 | 1-2 | 2-3 | 3-4 | 4-5 |
|---|---|---|---|---|---|
| K- | $0.00^{a} \pm 0.00$ | $0.00^{a} \pm 0.00$ | $0.00^{a} \pm 0.00$ | $0.00^{a} \pm 0,00$ | $0.40^{a} \pm 0.54$ |
| K+ | $1.40^{b} \pm 0.57$ | $2.80^{d} \pm 1.30$ | $6.00^{h} \pm 0.00$ | $6.00^{h} \pm 0,00$ | $6.00^{h} \pm 0.00$ |
| P1 | $0.00^{a} \pm 0.00$ | $0.40^{a} \pm 0.54$ | $1.00^{a} \pm 0.00$ | $1.00^{bc} \pm 0,54$ | $2.20^{cd} \pm 0.83$ |
| P2 | $0.00^{a} \pm 0.00$ | $1.20^{b} \pm 0.44$ | $2.00^{c} \pm 0.70$ | $3.20^{ef} \pm 0,44$ | $3.80^{f} \pm 0.44$ |
| P3 | $0.40^{a} \pm 0.40$ | $1.40^{b} \pm 0.54$ | $3.00^{de} \pm 0.70$ | $5.60^{gh} \pm 0.54$ | $5.80^{gh} \pm 0.44$ |

Note: Different superscripts show significant differences ($p<0.05$).
K- (Negative Control), K+ (Positive Control), P1 (Treatment 1), P2 (Treatment 2), P3 (Treatment 3)

**Table 2.** Mean and Standard Deviation of Mortality of *B. microplus* Adult Stage Based on Interaction of Treatment and Time

| Hopur / Treatment | 0-4 | 4-8 | 8-12 | 12-16 | 16-20 | 20-24 |
|---|---|---|---|---|---|---|
| K- | $0.00^{a} \pm 0.00$ | $0.00^{a} \pm 0.00$ | $0.00^{a} \pm 0.00$ | $0.00^{a} \pm 0.0$ | $0.20^{a} \pm 0.44$ | $0.40^{a} \pm 0.5$ |
| K+ | $0.40^{a} \pm 0.54$ | $1.60^{bc} \pm 0.8$ | $2.60^{a} \pm 1.51$ | $3.40^{f} \pm 1.1$ | $4.20^{gh} \pm 0.8$ | $4.60^{h} \pm 0.5$ |
| P1 | $0.00^{a} \pm 0.00$ | $0.00^{a} \pm 0.00$ | $0.00^{a} \pm 0.5$ | $0.40^{a} \pm 0.5$ | $0.40^{a} \pm 0.44$ | $0.80^{a} \pm 0.4$ |
| P2 | $0.00^{a} \pm 0.00$ | $0.20^{a} \pm 0.44$ | $0.80^{a} \pm 0.44$ | $1.40^{bc} \pm 0.5$ | $1.80^{cd} \pm 0.83$ | $2.20^{d} \pm 0.8$ |
| P3 | $0.00^{a} \pm 0.00$ | $0.60^{a} \pm 0.5$ | $1.20^{bc} \pm 0.4$ | $2.20^{d} \pm 0.4$ | $2.80^{e} \pm 0.83$ | $3.60^{f} \pm 0.5$ |

Based on probit analysis, it was shown that the LC50 of the ethanol extract of anting anting leaves against the larval stage of *B. microplus* was 4.05% and LC90 12.2%. This indicated that the low concentration of anting anting leaf ethanol extract was able to kill *B. microplus* larval stage in vitro. Probit analysis showed that the LC50 of the ethanol extract of anting anting leaves against the adult stage *B. microplus* was 6.8% and LC90 was 20.43%. The results of the analysis showed that to kill the adult stage of *B.microplus*, a much higher concentration of ethanol extract of anting anting leaves was required. Research on the potential of anting anting leaf extract against various parasites has been carried out, namely: *Anopheles stephensi* mosquito (Govindarajan *et al*., 2008), *Plasmodium falciparum* (Brahmam and Sunita, 2018), and *Sarcoptes scabiei* (Astuti, 2019). The results of these studies showed different lethal concentration results. Research by Govindarajan *et al* (2008) showed that the concentration of benzene extract of anting anting leaves which was able to cause death by 50% in third instar larvae of *Anopheles stephensi* was 19.25 ppm. The concentration of anting-anting leaf methanol extract which was able to cause death of 50% in third instar larvae of *Anopheles stephensi* was 15.3 ppm. The concentration of anting-anting leaf chloroform extract which was able to cause the death of the third instar larvae of *Anopheles stephensi* showed the highest value of 27.76 ppm. The results of Astuti's research (2019) stated that the LC90 concentration of the ethanol extract of anting anting leaves for the *Sarcoptes scabiei* was 12.5%. The results of Brahmam and Sunita's research (2018) showed that the chloroform extract of anting anting leaves was able to cause the death of 50% of *Plasmodium falciparum* at a dose of 1.47 g/mL. The concentration of ethyl acetate extract of anting anting leaves was able to cause 50% death of *Plasmodium falciparum* at a dose of 2.32 g/mL, while methanol extract required a larger dose to cause death of 50% in *Plasmodium falciparum*, which was 23.91 g/mL. The results of several studies can be concluded that anting anting leaf extract can be used as an antiparasitic with different doses depending on the type of parasite and the solvent used. Saponin compounds have soap-like properties and can damage the cuticle layer that protects the body of the *B. microplus* (Chaieb, 2010). The cuticle damage of ticks can facilitate the penetration of phytochemical compounds in other anting anting leaf extracts. Saponins are able to reduce the surface tension of the mucous membranes of the digestive system so that the walls become corrosive and damaged (Dinata, 2008). As a result, the work of digestive enzymes and food absorption in adult *B. microplus* can decrease. This mechanism can cause *B. microplus* to die because they are unable to digest food, starve and then become weak and die. Saponin compounds are also able to bind sterols in the digestive tract so that there is a decrease in the rate of sterols in the hemolymph (Muta'ali and Purwani, 2015). Sterols are precursors of the hormone ecdysone for the *B. microplus*. The decrease in sterol supply will greatly affect the ecdysone hormone in *B. microplus*. Ecdysone hormone is useful in the process of skin turnover in *B. microplus* to be able to grow to the next stage (Chaieb, 2010). The ecdysone hormone in adult female *B. microplus* also regulates the process of oogenesis and oviposition (Rees, 2004). This mechanism can cause *B. microplus* to die because they fail to metamorphose and oogenesis disturbances occur. The content of flavonoid compounds works by inhibiting acetylcholinesterase. As a result, acetylcholine cannot be broken down into choline and acetate. Inhibition of this enzyme can lead to accumulation of acetylcholine at the synapse. Accumulation of acetylcholine causes continuous stimulation of post synaptic receptors. The mechanism of action of flavonoids in the adult stage of *B. microplus* is less clear when compared to the larval stage of *B. microplus*. This mechanism indicates that the content of flavonoid compounds can act as neurotoxins in *B. microplus*.

## Conclusion

Anting anting leaf ethanol extract has potential as an acaricide against larval stage *B. microplus* and less potential as an acaricide in adult stage *B. microplus*.

## References

Abbas, R.Z., Zaman M.A., Colwell, D.D., Gilleard J., Iqbal, Z. 2014. Acaricide Resistance in Cattle Ticks and Approaches to its Management : The State of Play. J. Vet Parasitology: 6-20.

Adenubi, O.T, Fasina, F.O., McGaw, L.J., Eloff, J.N., Naidoo, V. 2016. Plant Extracts to Control Ticks of Veterinary and Medical Importance. South African J.of Bot: 178-193.

Astuti, L. T. 2019. Potensi Ekstrak Daun Anting-anting (*Acalypha indica* L.) sebagai Anti-Skabies terhadap *Sarcoptes scabiei var. cuniculi* secara*in-vitro* [Skripsi]. Fakultas Kedokteran Hewan, Universitas Airlangga.

Avinash, B.,Venu, R., Alpha, R.M., Srinivasa, R.K., Srilatha, C., and Prasad, T.N. 2017. *In-vitro* valuation of Acaricidal Activity of Novel Green Silver Nanoparticles Against Deltamethrin Resistance *Rhipicephalus (Boophilus) microplus*. J.Vet.Parasitol.1-24.

Brahmam, P. dan Sunita, K. 2018. Phytochemical Investigation and *In vitro* Antimalarial Activity of *Acalypha indica* L. and *Cocculus hirsutus* L. from Prakasam District, Andhra Pradesh, India. J.Biomed and Pharmacol. 2123-2134.

Chaieb, I. 2010. Saponin As Insecticide: A Review. Tunisian J. of Plant Protect.5: 39-50.

Dinata, A. 2008. Pemberantasan Penyakit Bersumber Binatang. http://www.litbang.depkes.go.id/lokaciamis/artikel/nyamuk-arda.htm.[20 Desember 2019]

Govindarajan, M., A. Jebanesan, T. Pushpanathan, dan K. Samidurai. 2008. Studies on the effect of *Acalypha indica* (*Euphorbiaceae*) leaf extracts on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). J. Parasitol. 691-695.

Hitchcock, L.F. 1955. Studies on the parasite stage of the cattle tick. *Boophilus microplus* (Canestrin)(Acarina, Ixodidae) Aust. J. Zoo. 3: 145-155.

Husna, S. 2014. Efektifitas Insektisida terhadap Larva Caplak (*Boophilus microplus*) Peternakan Sapi Potong di Jonggol Kabupaten Bogor [Skripsi]. Fakultas Kedokteran Hewan. Institut Pertanian Bogor.

Kumar, A.K., Sharma, A.K., Kumar, S., Ray, D.D., Rawat, A.K., Srivastava, S., dan Ghosh, S. 2016. Comparative *In-vitro* Anti Tick Efficacy of Commercially Available Products and Newly Developed Phyto-Formulation Againts Field Collected and Resistant Tick Lines of *Rhipicephalus (Boophilus) microplus*. J. Parasit. Dis. 40 : 1590-1596. Play. J. Vet Parasitology: 6-20.

Mohan, C.S., S. Dinakar, T. Anand, Elayaraja R., Sathiyapriya B. 2012. Phytochemical, GC-MS Analysis and Antibacterial activity of a Medicinal Plant *Acalypha indica*. Int. J. of PharmTech Res.1050-1054.

Muta'ali, R. dan Purwani, K. I. 2015. Pengaruh Ekstrak Daun Beluntas ( *Pluchea indica*) terhadap Mortalitas dan Perkembangan Larva *Spodotera litura* F. Jurnal Sains dan Seni ITS.4: 55-58.

Ress, H.H. 2004. Hormonal Control of Tick Development and Reproduction. J. Parasitol. 129(1):127-143.

Sindhu, Z., Jonsson N., Iqbal Z. 2012. Syringe test (Modified Larval Immersion Test): A New Bioassay for Testing Acaricidal Activity of Plant Extracts Againts *Rhipicephalus microplus* .J. Vet. Parasitol.188: 362-367.

Soulsby, E. J. 1982. Helminth, Arthropods and Protozoa of DomesticatedAnimals. London: 7th (hlm 462) Ed Billiere, Tindall & Cassel Ltd.

Wardhana, A.H., Amir Husein dan J. Manurung. 2005 . Efektifitas Ekstrak Biji Srikaya (*Annona squamosa* L) dengan Pelarut Air, Metanol, dan Heksan terhadap Mortalitas Larva Caplak *Boophilus microplus* secara *In-vitro*. JITV.10 (2) :134-142