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Ethanol Extract of Makassar Leaves (*Brucea javanica* [L.] Merr.) as a Potential Biopesticide against *Rhipicephalus sanguineus* in Dogs

(Ekstrak Etanol Daun Makassar (*Brucea javanica* [L] Merr) sebagai Potensi Biopestisida terhadap *Rhipicephalus sanguineus* pada Anjing)

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ABSTRAK

Rhipicephalus sanguineus adalah ektoparasit yang sering menyerang anjing, menyebabkan masalah kesehatan seperti anemia, iritasi kulit, dan penurunan berat badan. Penggunaan obat sintetis seperti pyrethroid efektif tetapi memiliki efek samping berbahaya bagi hewan, manusia, dan lingkungan. Oleh karena itu, penelitian ini bertujuan mengevaluasi ekstrak etanol daun makassar (*Brucea javanica* [L.] Merr.) sebagai biopestisida alami untuk mengendalikan *R. sanguineus*, mengingat kandungan tanin dan terpenoidnya yang berpotensi sebagai antiparasit. Penelitian ini dilakukan di Laboratorium Kesehatan Hewan Politeknik Pertanian Negeri Kupang dari bulan Mei sampai November 2022. Daun *Brucea javanica* diekstraksi menggunakan etanol 96% dan diuapkan hingga terbentuk ekstrak semipadat, kemudian diencerkan menjadi konsentrasi 10%, 20%, dan 30%, dengan kontrol positif Cypermethrin 100 g/L dan kontrol negatif aquades. Sebanyak 120 kutu dibagi ke dalam kelompok perlakuan dan diamati mortalitasnya setiap jam selama 8 jam. Data dianalisis menggunakan ANOVA dan uji lanjut Duncan. Hasil menunjukkan bahwa semua konsentrasi ekstrak (10%, 20%, dan 30%) menyebabkan mortalitas kutu yang signifikan ($P<0,05$) dibandingkan kontrol negatif, meskipun tidak berbeda signifikan dengan kontrol positif. Waktu penyemprotan optimal adalah 120 menit, dengan mortalitas tertinggi (1,80±2,111). Efektivitas ekstrak diduga berasal dari kandungan terpenoid yang mengganggu sistem saraf kutu dan tanin yang menghambat metabolisme. Namun, peningkatan konsentrasi tidak secara signifikan meningkatkan mortalitas, menunjukkan bahwa efek ekstrak mungkin telah mencapai titik optimal pada konsentrasi rendah. Ekstrak etanol daun *Brucea javanica* terbukti efektif sebagai biopestisida terhadap *R. sanguineus*, terutama pada waktu penyemprotan 120 menit. Namun, diperlukan penelitian lebih lanjut untuk mengisolasi senyawa aktif dan menguji efikasi pada dosis yang lebih tinggi serta metode aplikasi yang lebih baik. Temuan ini menawarkan alternatif alami yang potensial untuk mengurangi ketergantungan pada pestisida sintetis.

Kata Kunci : *Brucea javanica* (L.) Merr.; ekstrak herbal; mortalitas; *Rhipiecephalus sanguineus*

INTRODUCTION

Rhipicephalus sanguineus, a hematophagous tick, is an ectoparasitic tick that frequently infests dogs, causing detrimental issues (Merdana *et al*., 2020). Dogs with this parasite infection often exhibit alopecia, dermal irritation and infections, trauma, hematophagy leading to blood loss, anaemia (Bagas, 2020), weight loss, and a decrease in play with their owner (Nurcahya *et al*., 2020). This infestation often results in prolonged treatment, disease in the owners and their pets, and economic losses (Merdana *et al*., 2020; Munaf, 1986; Sudira, 2009). This parasite is also well-known as a biological vector of several important diseases, including botulism fever, babesiosis, ehrlichiosis, rickettsiosis, anaplasmosis, and Q fever (Dantas-Torres, 2010). These diseases in animals can cause minor to severe problems in a variety of organs, including the liver, lungs, heart, and nerves, ultimately leading to death. Therefore, it is important to tackle this issue.

Ectoparasiticides commonly used to treat ectoparasites in dogs include those containing pyrethroid synthetic compounds such as permethrin, Cypermethrin, and deltamethrin (Sudira, 2009) which is applied by spraying, injection, bathing-dipping, and dusting (Merdana *et al*., 2020). This medication is effective in eliminating ectoparasites that are present on the bodies of animals, including pets. However, extended use of this drug in pets, pet owners, and the environment may have detrimental effects. Various side effects that arise from treatment with this ectoparasitic drug include skin rashes, hypersalivation, seizures, skin allergies, irritation, watery eyes, vomiting, tremors, drug resistance, and, in severe cases, death (Nussa, 2016). Consequently, alternative therapies for tick infestations must be evaluated to mitigate chemical resistance in dogs and their effects on pet owners. In the environment, Cypermethrin can contaminate aquatic environments and possesses a high level of toxicity, posing a threat to aquatic organisms, bees, and birds (Tomlin, 1994).

Antiparasitic agents that are extensively used today are derived from herbal plants. *Brucea javanica* [L]. Merr. is a type of herbal plant that has been used for ectoparasite treatment due to its tannin and terpenoid content (Jacob *et al*., 2022; Jacob & Rumlaklak, 2020; Yasa & Jacob, 2024). Numerous studies have been conducted to evaluate the impact of herbal plants on the Rhipicephalus tick. Nonetheless, no research has yet been identified regarding the

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efficacy of *Brucea javanica* [L.] Merr. extract against ectoparasitic activity targeting the *Rhipicephalus sanguineus* tick. Therefore, this research aims to ascertain the anti-parasitic properties of *Brucea javanica* [L.] Merr. leaves extracts that can inhibit or eradicate *Rhipicephalus sanguineus* ticks on dogs.

MATERIALS AND METHODS

The study was performed from May to November 2022 at the Animal Health Laboratory, Department of Animal Husbandry, Kupang State Agricultural Polytechnic. The leaves of the makasar plant were sourced from Kuanoah small village in Noelbaki, Kupang Tengah District. The samples were collected at an elevation of 10-20 meters above sea level. The equipment that was utilized consists of a pyrex's beaker glass, an analytical balance, a rotary evaporator (®Heidolph Hei-VAP Gold1, Germany), a water bath, a mortar, a Miyako blender, a 65 mesh sieve, a stamper, a spatula, parchment paper, a porcelain dish, an ose, a spatula, a spirit lamp, an electric balance, a hotplate, a calliper, a coarse cloth, tissue, an ointment tube, a round glass with a diameter of 15 cm, sterile gauze, a glass jar, a pH meter (®AS 218), an object glass, and gloves.

The makassar leaf samples were extracted for research through multiple processes. The extraction phases in this research relate to the methodology outlined by Merdana *et al*., (2020). The leaves are harvested and desiccated at ambient temperature until fully dry. Upon drying, the leaves are pulverized into a fine powder, yielding 120 grams of simplicia. The powder is subsequently immersed in 96% ethanol at a 1:5 (w/v) ratio within a sealed glass vessel. The soaking solution is maintained at room temperature for three days, with constant stirring and repetition occurring six times. Filtration is performed to acquire the filtrate, which is subsequently evaporated at 70 °C until a viscous semisolid extract is produced (Zhang *et al*., 2018).

The semisolid extract derived after evaporation is subsequently diluted to the specified treatment concentrations. This study has five treatments: The positive control was Cypermethrin 100 g/L (K+), the negative control was aquades (K-), and there were three treatment groups: 10% extract (K1), 20% (K2), and 30% (K3). A 10% concentration solution was prepared by weighing 10 grams of Makassar leaf extract, transferring it to a volumetric flask, adding aquades to achieve a final volume of 100 mL, and mixing until

homogeneous (Roman *et al*., 2021). The 20% and 30% solutions were made in the identical approach. After homogenization, the extract solution was transferred into a 100 mL spray container. The positive control group utilized Cypermethrin at a concentration of 100 g/L, combined with aquades as per the specified dosage, and administered via a 100 mL sprayer. Each treatment group received 10 sprays of the plant extract every 30 minutes, and observations continued until the ticks were deceased. Similarly, the control group received Cypermethrin for the same length as the treatment group.

This study used a total of 120 *R. sanguineus* ticks, evenly distributed among the groups. One group included four ticks with five repeats, and the sample size was determined using the Federer formula (t-1) (n-1) ≥15. Morphological tick identification was done after the collection and before the treatment. Ticks are arranged in petri dishes in accordance with the quantity of each group. Then, 5 grams of cotton are taken and sprayed with the treatment material for the group. This cotton is then deposited in each petri dish containing ticks. Each petri dish is subsequently observed from minute 0 to minute 480 following the placement of the cotton. If the tick cannot revert to its previous position or exhibits no movement, it is deemed deceased (Merdana *et al*., 2020).

This study used a completely randomized design (CRD) for data analysis. The data is subsequently examined utilizing the Analysis of Variance (ANOVA) test, followed by the LSD and Duncan tests to examine the interaction among the treatments.

RESULTS AND DISCUSSION

Table 1 presents the average mortality rate of ticks at each extract concentration (K1-K3), demonstrating a death rate that is not substantially different (P>0.05) from the positive control (K+), but is considerably different from the negative control. This table indicates that the lowest tick mortality rate is observed at K1 (0.71±1.23), although K2 and K3 exhibit identical values (0.75±1.48 and 0.75±1.42, respectively). The table reveals that, despite variance in the mortality rate, the increase in extract concentration from 10% to 30% does not significantly affect tick mortality.

The extraction results indicate that the treatment group demonstrated more pronounced effects than the negative control, implying that the active constituents in this biopesticide

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possess insecticidal properties against the tick *Rhipicephalus sanguineus*. This result suggests that the efficacy of the active constituents in the extract may have reached an optimal level at a specific quantity. This study's findings contrast with those of Soares *et al.* (2019), which asserts that the toxicity of biopesticides is exactly proportional to the concentration of active components in a solution. In addition, Pramadaningtyas *et al.* (2023) and Soares *et al.* (2019) conclude that an increased concentration of active components in a solution correlates with heightened toxicity of the biopesticide, hence enhancing its efficacy in extermination.

Table 1. Average tick mortality for each treatment

Group	Average Number of Tick Deaths
K-	0.00±.000[a]
K+	0.75±2.027[b]
K1	0.71±1.233[b]
K2	0.75±1.482[b]
K3	0.75±1.422[b]

Desription: K-: negative control aqua; K+: positive control Cypermethrin; K1: 10% concentration of *Brucea javanica* [L.] Merr. leaf extract; K2: 20% concentration of *Brucea javanica* [L.] Merr. leaf extract; K3: 30% concentration of *Brucea javanica* [L.] Merr. leaf extract
Different superscripts a,b on the same column indicates a significant difference (P<0.05)

Table 2 illustrates that the average mortality rate of ticks at various spraying intervals of the extract reveals that the ideal spraying period for tick eradication is 120 minutes, yielding the highest average mortality rate of 1.80±2.111. The research suggests that a spraying period of 120 minutes is the most effective for exterminating ticks. Conversely, alternative spraying durations, ranging from 180 to 420 minutes, exhibit negligible efficacy, with an average death rate nearing zero (P<0.05). This suggests that the efficacy of spraying is affected by the contact period between the spray solution and the ticks. With a length of 120 minutes, the active ingredient functions optimally; however, extended durations may diminish its efficacy due to potential instability or degradation. This supports the assumption that the efficacy of pesticides is significantly dependent upon the ideal contact duration to attain maximal penetration (Lee *et al.*, 2019).

The administration of *Brucea javanica* [L.] Merr. plant

extract as a biopesticide will decelerate the mortality process in ticks, as it operates gradually; yet, it may induce stress, alter digestion, result in fluid depletion, and ultimately lead to the demise of ectoparasites in contrast to chemical pesticides. Herbal plant extracts can directly harm the tick's nervous system; however, many herbal components may not exhibit a significant fatal effect but rather interfere with the tick's metabolism (Nussa, 2016).

Table 2. Mean tick mortality at each time of extract application

Tick Spraying Time	Tick Mortality Rate
0 Minutes	0.00 ±.000[a]
60 Minutes	1.20 ±2.484[c]
120 Minutes	1.80 ± 2.111[d]
180 Minutes	0.53 ±0.915[ab]
240 Minutes	0.80 ±1.265[bc]
300 Minutes	0.40±0.828[ab]
360 Minutes	0.00±0.000[a]
420 Minutes	0.00±0.000[a]

Description: [a,b,c,d] Distinct superscripts on the same line denote significant differences (P<0.05)

The phytochemical analysis of the leaves from the plant sourced in Noelbaki Village indicates the presence of terpenoids and tannins as its secondary metabolites (Yasa & Jacob, 2024). Terpenoids are substances prevalent in numerous plants that possess the capability to exterminate ticks by interfering with their nervous system, whereas tannins affect tick metabolism and can bind proteins, particularly during digestion (de Melo *et al.*, 2023). Moreover, Bagas (2020) asserts that tannins are chemicals that contribute to a plant's defensive mechanism against insects and are present in nearly all green plants globally. This chemical functions by activating insect proteolytic enzymes, leading to cell lysis and subsequent insect cell death (Bagas, 2020). This substance binds to proteins in the tick's digestive system, consequently impeding the insect's digestion during its growth. This aligns with the assertion that tannins behave as antifeedants, as these substances impede intestinal action, leading to a lack of energy production due to inefficient metabolism (Susanti, 2019; Yunita *et al.*, 2009).

CONCLUSION

This study's findings demonstrate that the extract of *Brucea javanica* [L.] Merr. functions as an antiparasitic agent capable of exterminating the *Rhipicephalus sanguineus* tick on dogs, provided

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that the timing and concentration are precise. Consequently, additional studies are required to employ methodologies that isolate the active compounds functioning as antiparasitic to eliminate ticks on animals.

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