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Research Article

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Bay leaves (*Syzygium polyanthum*) extract as a repellent of *Culex quinquefasciatus*

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ABSTRACT

The study of bay leaf extract had been done to know its potential as *Culex quinquefasciatus* repellent. Bay leaf extract has the secondary metabolite compounds, such as terpenoid that is known as repellent of mosquitoes. The aim of this study was to determine the effect of bay leaf extract on Culex quinquefasciatus. The method used in this study was experiment with complete randomized design which was consisted of six treatments and four repetitions. The treatment consisted of control (0%), P1 (10%), P2 (20%), P3 (30%), P4 (40%), and P5 (50%) of bay leaf extract. Data were analyzed by using one-way ANOVA. The results of this study showed the lowest mean of *C. quinquefasciatus* which repelled was 83% and the highest was 95,5%. Analysis of variance indicated that amount of *C. quinquefasciatus* which avoided bay leaf extract was significantly different. Based on the result, it can be concluded that bay leaf extract is potential as *C. quinquefasciatus repellent*.

Keywords: *Culex quinquefasciatus*; repellent; *Syzygium polyanthum*

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INTRODUCTION

Bay leaf is part of plant which is widely used as Indonesian seasoning, mainly in Sumatera, Java, and Bali (Batool et al., 2020; Mudiana, 2016). Bay leaf is also used as traditional medicine (Geck et al., 2020; Guzmán et al., 2014; Jit Singh & Kumar Thakur, 2014; Putri, 2016; Zulcafli et al., 2020). Moreover, bay leaf extract contains terpenoid, consisted of sesquiterpenes (*β–cariopillen, α–pinen, eugenol, linalool*) (Fidan et al., 2019; Putri, 2016), known as repellent of mosquito (Geetha & Roy, 2014; Tawatsin et al., 2006; Wooding et al., 2020; Wu et al., 2015; Zhu et al., 2018).

Repellent is compound which prevent mosquito for flying, descending, or piercing (Campos et al., 2020; Dahmana & Mediannikov, 2020; Ranasinghe et al., 2016). Generally, synthetic repellent is widely used in society due to its effectiveness, helpfulness, and profit for human used. Nevertheless, long-term synthetic repellent utilization causes negative effect to skin such as nausea, vomit, central nervous system disorder, even death due to the N-diethyl-m-toluamide/DEET (Chen-Hussey et al., 2014; Syed & Leal, 2008). Hence, natural repellent is required for better prevention. Natural repellent is simple because it is easy to produce and relatively effective for human protection. Several studies showed that terpenoids is a natural repellent of mosquito.

Study about repellent activity of terpenoids from several plants shows a repellent effect for *Anopheles stephensi* (ethanol extract of neem (*Azadirachta indica*) (K Murugan et al., 2014), *Aedes aegypti* (*Cinnamomum cassia*, *Cinnamomum camphora*, *Paeonia suffruticosa*, *Nardostachys chinensis* rhizome (Yang et al., 2004), both *Aedes aegypti* and *Anopheles arabiensis* (*Apium graveolens* and *Tagetes minuta*) (Kumar et al., 2014; Wanzala & Ogoma, 2013). Furthermore, sesquiterpenes from several plants also shows repellent effect for yellow fever mosquito, *Culex tritaeniorynchus*, *Anopheles subpictus* (*Cymbopogan citratus, Cinnamomum zeylanicum, Rosmarinus officinalis, Zingiber officinale, Amyris balsamifera*, *Fokienia hodginsii* (Govindarajan, 2011; Paluch et al., 2009) and *Culex quinquefasciatus* (*Zingiber officinalis* (zingiberol and β–eudemol) (Pushpanathan et al., 2008)). However, aromatic essential oils of bay leaf (*Syzygium polyanthum*) are expected as mosquito's repellent, especially *C. quinquefasciatus,* and have not scientifically reported yet.

C. quinquefasciatus is vector of filariasis' disease (Manyi et al., 2014; Kadarkarai Murugan et al., 2015) such as lymphatic filariasis or elephanthiasis which is particularly caused by *Wuchereria bancrofti* and *Brugia malayi* (Cranston and Gullan, 2005). *C. quinquefasciatus* is nocturnal mosquito and it often disturbs human in sleeping. Consequently, mosquito repellent is required for prevention of filariasis disease. Bay leaf extract is suggested to play role as mosquito repellent of *C. quinquefasciatus* and it has not been scientifically reported yet. Therefore, the further study about bay leaf extract (*Syzygium polyanthum*) as *C. quinquefasciatus'* repellent is required.

RESEARCH METHODS

S. polyanthum were collected from Prabumulih, South Sumatra. The leaves used were the third to the fifth strand from the tip of main leaf stalk. Fresh leaves of *S. polyanthum* was collected, washed, dried in room temperature. The dried leaves were powdered by using a blender. Then, it was macerated in 96% methanol for 3 days to obtain crude extract. Afterward, the crude extract was filtered by using filter paper (Whatmann). Concentrated extract was obtained by evaporate the methanol using rotary evaporator. The concentrated extract was stored at 4 °C for research use (Begashaw et al., 2017). Terpenoids were monitored by using n-hexane:chloroform (0:10) with Cerium (IV) Sulphate spray reagent. Sample contains terpenoid if brownish red color is formed on TLC (Thin Layer Chromatography) (Sinurat et al., 2020).

Larva of *C. quinquefasciatus* was identified by using atlas of mosquito morphology (Michele M. & O'Meara, 2008). Larva instar III was used in this study. Larva identified was included to container as many as 50 individuals per cage in total 24 cages (23 x 23 x 50 cm^3). Larva was kept in to the cage until imago and was acclimatized for three days. Mice was put in the cage as food source. This study used complete randomized design which consisted of six treatments and four repetitions. The treatments consisted of 0% (P0), 10% (P1), 20% (P2), 30% (P3), 40% (P4), and 50% (P5) of bay leaf extract. Repellent test used mosquitrap. Mosquitrap used was made by using mineral water bottle (1,5 L

volume) which was cut 2/3 of cap. Glue was applied on the inside and outside part of mosquitrap (De Santos et al., 2012). This trap was consisted of bay leaf extract and placed in the cage filled imago (start at 5.30 pm) for one night. We used the numbers of mosquitoes which were not trapped as the data and analyzed by using one-way analyses of variance (ANOVA). If the results of the analysis of variance show very significant different results, then proceed with the Honestly Significant Difference (HSD) test.

FINDING AND DISCUSSION

Based on observation, there was mean variation of *C. quinquefasciatus* which repelled in the mosquitrap (Figure 1). This variation showed that bay leaf extract was potential as *C. quinquefasciatus* repellent. The mean of *C. quinquefasciatus* which repelled in the mosquitrap varied in each treatment compared to control. The highest percentage of *C. quinquefasciatus* which repelled the mosquitrap was 95,5% while the lowest percentage was 83%.

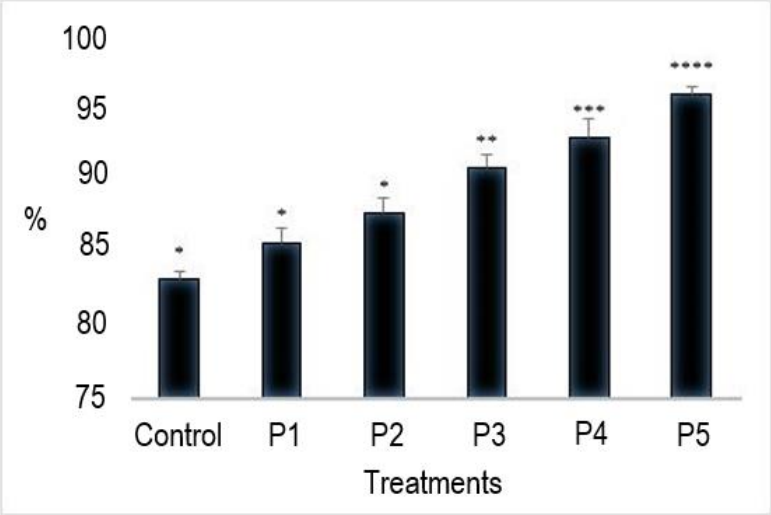

Figure 1. Mean of *C. quinquefasciatus* repelled

Analysis of variance (ANOVA) showed that effect of bay leaf extract was significantly different in repelling *C. quinquefasciatus* ($F=26.166$, $p>0.01$). It means that bay leaf extract has a role as repellent of *C. quinquefasciatus*. Thus, the Tukey test (HSD) is required to examine the effect of bay leaf extract as repellent of *C. quinquefasciatus*.

The HSD results showed that P5 was significantly different to other treatments. However, P3 was an optimum concentration due to the same effect with P4 and P5 (Figure 1). Treatment with the highest concentration of bay leaf extract showed the highest percentage of *C. quinquefasciatus* repelled. Based on the study, the effect of bay leaf extract in repelling *C. quinquefasciatus* significantly was showed by P3, P4, and P5.

This study demonstrated that concentrations of bay leaf extract had effect as repellent of *C. quinquefasciatus*. HSD test showed that P1 and P2 had equal effect with control (P0) while effect of bay leaf extract was significantly demonstrated by P3, P4, and P5 (Figure 1). Thus, the higher concentration of bay leaf extract, the more mosquitoes to avoid. It is supposed that bay leaf extract contains several aromatic compounds, such as terpenoid which has role as mosquito's repellent. Based on thin layer

chromatography (TLC) test, bay leaf extract formed brownish red (Figure 2). It was indicated that the extract contained terpenoid.

Figure 2. Terpenoid on TLC analysis

Figure 2 showed that the bay leaf extract contained terpenoid group compounds, such as monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, or tetraterpenoids with the appearance of a brownish-red spot (Online et al., 2020). However, it still requires further research to determine the exact compounds. Based on the references, terpenoid in bayleaf extract which roles in repeletion is *β–cariopillen, α–pinen, eugenol, linalool.* Terpenoids are volatile compounds (Dudareva et al., 2013; El-Zaeddi et al., 2016). The stability of essentials oil depends on concentration, storage time, and temperature. The longer it is exposed, the less volatile molecules will be appeared (Jesus et al., 2016). Thus, the higher concentration of bay leaf will lead to longer disappearance volatile molecules. It was suggested that the higher concentration of *S. polyanthum* extract, the more number of *C. quinquefasciatus* repelled.

C. quinquefasciatus has a flagella in its antenna. The flagella has detector called sensilla trichodea which is devided to five subtypes, specifically long sharp pointed-tipped (LST), short sharp-tipped (SST), short sharp-tipped curved (SSTC), short blunt-tipped type I (SBT I), and short blunt-tipped type II (SBT II). The subtypes of sensilla have their respective function not only in olfactory sensitivity but also nervous pattern activity to respon various volatile molecules (Fan et al., 2011; Hill et al., 2009). In this study, several compounds suggested as repellent were linalool, eugenol, α–pinene, and β–cariophyllene (Guo et al., 2016; Kim et al., 2016; Medeiros et al., 2013; Nerio et al., 2010). The suggestion about repelletion mechanism of *C. quinquefasciatus* needs further information. However, it is predicted that it go through ORNs of *C. quinquefasciatus* which has specific function. Each volatile molecule passing through the sensilla trichodea pores, such as linalool, eugenol, and α–pinene, can be detected by SBT II, SBT I, and SST, respectively (Hill et al., 2009). Nevertheless, β–cariophyllene is still unknown yet. It act as stimulant which react to receptor (G protein) in plasma membrane and it activate two-ways signal.

G protein releases one of subunit, namely Gα-GTP which induces adenyl cyclase for cAMP synthesis and c-phospholipase for synthesis inositol 1,4,5-triphoshate (IP3). cAMP, as second messenger, will affect stability of Ca^{+2} and membrane potential while IP3 difuses to sitoplasm and reacts to Ca^{+2} channel in reticulum endoplasm. It causes Ca^{+2} into sitoplasm of ORNs and induces membrane

depolarization and produces signal to be forwarded to axon central nervous system (Medeiros et al., 2013; Zwiebel & Takken, 2004).

Signal in terminal axon causes membrane depolarization so calcium can enter it. Calcium induces glutamate, as neurotransmitter, into synaptic gap. Glutamate binds to AMPA and NMDA receptor in postsynapstic membrane. Reaction of glutamate and AMPA will open Na^+ channel. Na^+ causes depolarization of postsynaptic membrane and produces Excitatory Postsynaptic Potencial (EPSP). Reaction of glutamate and NMDA will open Ca^{+2} channel, but it cannot come in due to blocking Mg^{+2}. The way can be opened by depolarisation produced by reaction of glutamate and AMPA receptor so Ca^{+2} can come in. Ca^{+2} activates cAMP channel in postsynaptic that causes insersion of AMPA and cells release paracrin retrograde, nitrit oxide. Nitrit oxide will difuse to prasynaptic neuron to increase glutamat (Medeiros et al., 2013). This mechanism plays role to remember the molecules accepted by mosquito and the brain will instruct motoric, wings, and legs, to avoid.

CONCLUSION

Based on the study, it can be concluded that *Culex quinquefasciatus* avoided due to the influence of bay leaf extract. Thus, bay leaf extract has the potential as repellent.

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