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Toxicity of Miana Leaf (*Coleus blumei*) Extract Against Houseflies (*Musca domestica*)

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Abstract

Houseflies (*Musca domestica*) is an disease-carrying insect that plays an important role in the spread of disease in humans and animals. One way to control houseflies is using Miana leaves (*Coleus blumei*) as botanical pesticide. The purpose of this study was to determine the chemical compounds contained in Miana leaf as well as determine the optimal concentration of Miana leaf extract in causing *knockdown* effect in houseflies. Miana leaf fine powder was soaked with methanol (maceration) for 3 days followed by phytochemical screening (chemical reagents and Gas Chromatography Mass Spectrometry) and anti-houseflies toxicity tests. Testing of methanolic extract of Miana leaves was made in the form of spray using the knockdown method at concentrations of 5%, 10%, 20%, 30% and 40% and observed in 5, 10 and 15 minutes. The results of phytochemical screening with chemical reagents showed that the extract contained alkaloids, tannins, flavonoids, saponins and terpenoids; while the results of GCMS analysis showed 8 bioactive compounds. The results of the study revealed that at a concentration of 30% and 40%, methanolic extract of Miana leaves could result in a *knockdown* effect that was accompanied by death with a percentage value of 100% in the first 5 minutes of observation. This research shows that methanolic extract of Miana leaves can be used as an alternative to botanical pesticides in controlling houseflies (*Musca domestica*).

How to Cite

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INTRODUCTION

Houseflies (*Musca domestica*) is one of the insect members of the Diptera order (family Muscidae). They are ubiquitous and able to live in all environment; this makes houseflies cosmopolitan insects (Hastutiek & Fitri, 2007). These insects generally live and often move to a dirty environment, thus carrying various kinds of pathogenic disease-carrying microorganisms. Diseases caused by houseflies include typhus, cholera, diarrhea, parasitic worms, dysentery, trachoma viruses and other digestive infections (Ibrahim et al, 2018).

The most common way to control insect pests (flies) is through the use of synthetic pesticides. However, it has a negative impact on the environment (Gaag, 2000), such as accumulation of chemical residues in the environment and food chain that will disrupt the ecosystems (Damalas & Eleftherohorinos, 2011), water and food contamination (Soares & Porto, 2009) as well as pests resistance (Cole et al, 2000).

To overcome this problem, it is necessary to be able to control flies using natural materials, such as by utilizing the plants (biopesticides). According to Elbermawy et al (2011), plants are used as botanical pesticides because they contain various kinds of chemical compounds that have toxic effects on insect pests, such as *repellent* (insecticidal), *antifeedant* (disturbing diet) and the one that disturbing insect growth. Using plants as botanical pesticides have several advantages because raw materials (plants) are easy to get, nontoxic, and their compounds are biodegradable and safer for humans, food and the environment (Moreira et al, 2007).

Miana (*Coleus blumei*) is a member of the Lamiaceae family. This plant is better known as Mayana and is commonly used as an ornamental plant because it has attractive and striking leaf colors. Miana leaves contain alkaloids, tannins, flavonoids, saponins and terpenoids so that the leaves of Miana are widely used as a remedy for asthma, coughing, bronchitis, neutralizing toxins, diarrhea and worm medicine (Wijayakusuma, 2005). Because Miana leaves contain many of these chemical compounds, therefore this research uses Miana leaves as a houseflies *repellent*.

Yuliana et al's research (2016) shows that the content of alkaloids, flavonoids and saponins in the frangipani leaves (*Plumeria acuminate*) is able to kill the house flies (*Musca domestica*) by 77% at a concentration of 60%. Along the similar line, Singh & Kaur's research (2016) shows that the flavonoid content of *Ricinus communis* leaves is able to kill the houseflies. Whereas Fitriana research (2014) demonstrates that the essential oil content in purple basil leaves (*Ocimum sanctum*) has the potential as larvacide against *M. domestica* larvae with a mortality of 52% at a concentration of 20%.

However, the study of Miana (*Coleus blumei*) leaf as a botanical pesticide against houseflies (*Musca domestica*) has not been done before. Therefore, this study was conducted to determine the chemical compounds of methanolic extract of Miana leaves and its toxicity against houseflies (*Musca domestica*) using the *knockdown* method.

This study aims to explore the benefits of Miana leaves as a potential insect pest control. It demonstrates that the Miana leaves can be used as an alternative botanical pesticides as anti-houseflies. It is the kind of *repellent* that is cheap and environmental friendly. It helps to increase the utilization of the Miana plants, which is commonly regarded as the ornamental plants

METHODS

The materials used in this study included Miana leaves, methanol solvents, houseflies (*Musca domestica*), filter paper and chemical reagents. While the tools used were glass jars (for maceration), funnels, analytical scales, stirring rods, flies test jars, blenders, glassware, dropping pipettes, measuring pipettes, stoves and Gas Chromatography Mass Spectrometry.

Miana plant samples (Figure 1) were obtained from the Juanda area of Sidoarjo, East Java. Then the plant was identified at the Biology Service Unit of Airlangga University in Surabaya to ensure that the plant was a Miana plant (*Coleus blumei*).

Figure 1. Miana (*Coleus blumei*) plants

Extraction process of Miana leaves

Extraction was done by maceration method. Miana leaves were washed, then dried, cut

into small pieces and blended until became a fine powder. As many as 20 grams of fine powder of Miana leaves were put in a glass jar, then added with 600 ml of methanol solvent. Soaking (maceration) was carried out for 3 days, then the solution was filtered using filter paper and funnel. During maceration, stirring was carried out every day to avoid the compacting of the fine powder. Maceration process was carried out 2 times.

Chemical Compounds Screening of Miana Leaf Methanolic Extract

One hundred ml of extract was taken for phytochemical screening process with reagent and GCMS to determine what chemical compounds contained in the extract. Phytochemical screening with chemical reagent to determine the presence of alkaloids, tannins, saponins, flavonoids, and terpenoids was carried out at the Pharmacognosy Laboratory of the Surabaya Pharmacy Academy. While the bioactive compound test with GCMS was conducted at the Pharmacy Unit Service of Airlangga University.

Making the Various Concentration of Miana Leaf Extract

The concentrations used in the toxicity test were 5%, 10%, 20%, 30% and 40%. In order to get a concentration of 5%, 5 ml of extract was added with 95 ml distilled water and put in a 100 ml sized spray bottle.

Toxicity Test of Miana Leaf Extract Against Houseflies

The toxicity test of houseflies using the *knockdown* method was carried out in accordance with the research conducted by Kardinan (2007) with several modifications (Figure 2). Holes were made in the lid of the test jar for air circulation so that the houseflies did not die, and the other holes were made to spray the Miana leaf extract.

Each test jar which had been labeled according to the predetermined concentration contained 25 houseflies. Then, the extract was sprayed 2 times (0.5 ml for each spray). The observation was then conducted to count the knocked down houseflies every 5, 10 and 15 minutes. The treatment was carried out with 3 replications.

Calculation of Knocked Down Houseflies

The percentage of houseflies that experienced *knockdown* was calculated using the equation from Kardinan (2007) as follows:

% Knockdown = (Number of knocked down flies)/(Number of initial flies) x 100%

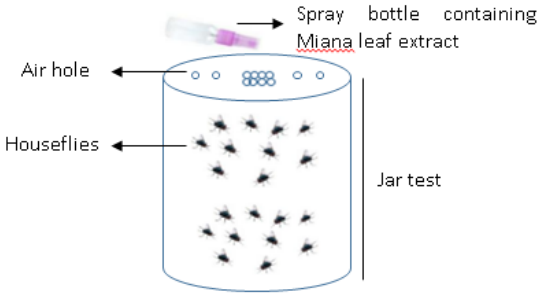

Figure 2. Toxicity test of methanolic extract of Miana leaf against houseflies by *knockdown* method

RESULTS AND DISCUSSION

Phytochemical Screening

The results of phytochemical screening with chemical reagents showed the alkaloids, flavonoids, tannins, saponins and terpenoids content (can be seen in Table 1). While, Table 2 shows the content of chemical compounds analyzed by GCMS, i.e. (2,4,4,4,16,16-D6)-3.alpha., 17.beta.-dihydroxy-5.beta.-androstane; (E,E)-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol acetate; 1,8-bis(3,4-dicyanophenyl)anthracene; 23-R-methylcholesterol; Stigmasterol; Stigmast-8(14)-en-3.beta.-ol; and .alpha.-Amyrin acetate.

Toxicity Test

Concentrations of extract used in this study were 5%, 10%, 20%, 30% and 40%. The method used was *knockdown* method, the samples were observed for 5, 10 and 15 minutes. Each test

Table 1. Results of chemical compounds analysis of Miana leaf methanolic extract with chemical reagents

Chemical compounds	Test Method	Result	Observation
Tannin	FeCl3 1 %	+	Greenish black colour
Alkaloid	Dragendorff Test	+	Orange red precipitate
	Mayer's Test	+	Dull white precipitate
Saponin	Foam Test	+	Formation of 1 cm layer foam
Flavonoid	Lead Acetate Test	+	Yellow colored precipitate
Terpenoid	Salkowski's Test	+	Reddish brown ring

Table 2. Results of chemical compounds analysis of Miana leaf methanolic extract with GCMS

Peak	RT	Total (%)	Name of compound	Biological activities
1	20.88	7.18	(2,4,4,4,16,16-D6)-3. alpha.,17.beta.-dihydroxy-5.beta.-androstane	antiproliferative (Selvamangai & Bhaskar, 2013)
2	25.45	4.97	(E,E)-3,7,11-trimethyl-2,6,10-Dodecatrien-1-ol acetate	antibacterial, antifungal (Chandrasekaran et al, 2005), thickener, moisturizer and softener (Lide, 2005), as a nutritious substance and metabolite in living organisms (Cakir, 2004)
3	27.52	5.02	1,8-Bis(3,4-dicyanophenyl)anthracene	antioxidants (Traber & Manor, 2012), anti-inflammation, antibodies (Rizvi et al, 2013)
4	28.14	13.35	23-R-methylcholesterol	anti-inflammation (Mahatheeranont et al., 2015), antibacterial, antifungal (Chaveerach et al, 2017), cytotoxic against prostate, breast, lung, gastric and ovarian cancer cells (Choi et al, 2007; McCann et al, 2003; De Stefani et al, 2000)
5	28.33	24.75	Stigmasterol	reduce the risk of cardiovascular disease (heart disease) (Ferrer et al, 2017), food additives (EFSA, 2012), precursors of sex hormones and vitamin D3 precursors (Mondul et al, 2011), anti-angiogenic (Kangsamaksin et al, 2012)
6	28.80	28.71	Stigmast-8(14)-en-3. beta.-ol	anti-osteoarthritis, anti-hypercholesterolemia,antitumor, antioxidant and anti-inflammation (Chaudhary et al, 2011)
7	29.21	16.04	.alpha.-Amyrin acetate	anti-inflammation, antibacterial, antifungal, antiviral, anticancer (Palazon et al, 2012), analgesics (Hernandez et al, 2010)

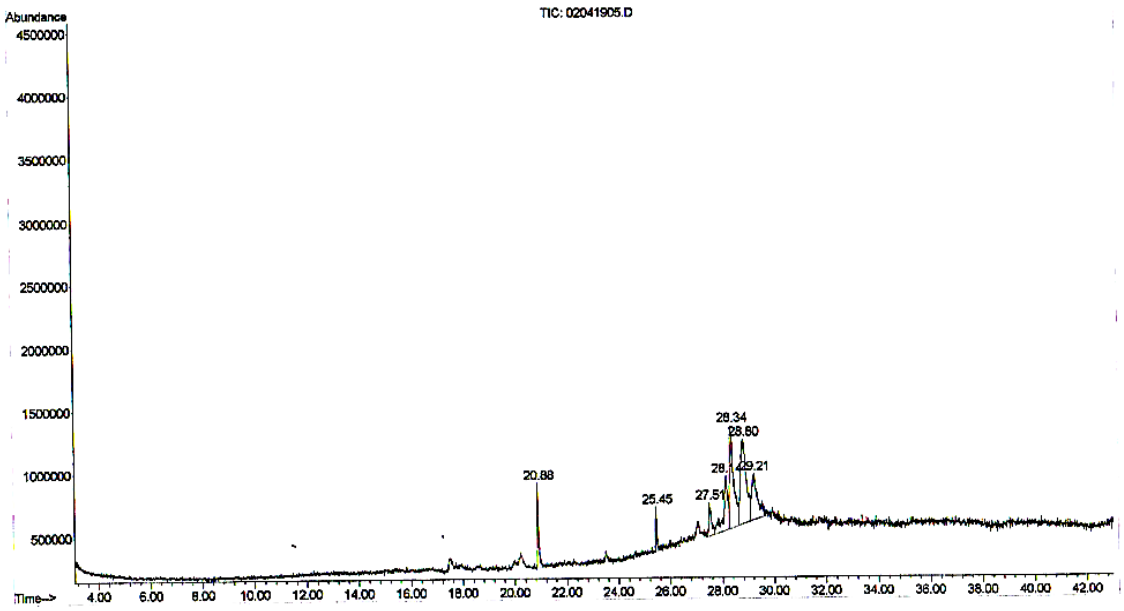

Figure 3. Results of the GCMS chromatogram of methanolic extract of Miana leaves

jar contains 25 houseflies. The results of the toxicity test of Miana leaf extract are presented in Table 3 as follows:

Table 3. Results of toxicity test of Miana leaf methanolic extract against houseflies

Time (minute)	Knockdown (%)				
	5%	10%	20%	30%	40%
5	36	32	72	100	100
10	60	92	100	100	100
15	92	100	100	100	100

The effectiveness of Miana leaf extract as a botanical pesticide is indicated by the percentage of *knockdown* houseflies based on the specified time interval. The higher the percentage, the higher potential of extract as a botanical pesticide.

Based on the method used, the vapors that contain insecticidal chemical compounds entered the body of the houseflies through the respiratory system. That method results in poisoning that occurs in the respiratory system and affects the nervous system in houseflies (Wirawan, 2006). This is in accordance with the statement of Sumita et al (2016), where spiracles located on the body surface of insects play an important role as a cause of *knockdown* in housefly. Vapor extracts enter the spiracles and quickly enter the insect nervous system, then inhibit the action of the acetylcholinesterase enzyme and finally a *knockdown* occurs.

In Table 3, it can be seen that the increasing concentration of Miana leaf extract was followed by the increase in the percentage of *knockdown* in *Musca domestica*. The higher concentration of extract, then the more chemical compounds contained in it, thus it will shorten the life span of test insects.

The results of this study were supported by Darmadi & Anita (2018), which showed that 15% concentration of Duku (*Lansium domesticum*) peel extract spray was able to kill *Musca domestica* within 12 minutes. The results of the study by Yuliana et al (2016), stated that *Plumeria acuminate* leaves extract spray can killed house flies by 76.7% at a concentration of 60%. Whereas Kumalasari et al (2015) stated that electric mat vaporizer containing Kesum (*P. minus*) leaf methanol extract at a concentration of 25% resulted in 75% of *knockdown* of the housefly (*Musca domestica*).

Result of toxicity test showed that all concentrations of Miana leaf extract in the first 5 minutes gave a real *knockdown* effect, especially at concentrations of 30% and 40%, where the number of houseflies fell (*knockdown*) accompanied by death was 100%. At the concentration of the Miana leaf extract of 5% and 10%, there is an increase in the number of houseflies that fall. However, when the concentration level is 5%, this experiment shows a number of flies which still can be recover by 8%. These *knockdown* flies are still alive; they would respond to any moving and touching triggers. For example, they would make a response to a brush stroke. At the concentration of the Miana leaf of 20%, the effectiveness is almost equal with the one found at the concentration of the Miana leaf of 30% and 40%.

The ability of methanolic extract of Miana leaves as an insect *repellent* (ie anti-houseflies) is thought to be due to the presence of secondary metabolites which are toxic to insects. The results showed that the methanolic extract of Miana leaves contained alkaloids, tannins, saponins, flavonoids and terpenoids. This is supported by research conducted by Yuliani et al (2005), where alkaloid and terpenoid compounds in *Pogostemon cablin's* methanol extract have *repellent* effect against *Musca domestica*.

Alkaloid compounds are included in toxic compounds. It works through attacking the works of the insect's nervous system. Such attack will slow down the nervous system, create seizure and *knockdown* and leads to death. Flavonoids are also toxic to insects by weakening the nervous system in spiracles (breathing apparatus in insects) found on the surface of the body, so that the spiracles become damaged. As a result, insects cannot breathe and eventually die (Yuliana et al, 2016). Saponins and terpenoids damage the cell membranes and disrupt insect metabolic processes resulted in the death of insects (Heras & Hortelano, 2009). Tannin damages the permeability of insect cell membranes, so that it disrupts the metabolic process and the insect will eventually experience death (Purwita et al., 2013).

The chemical compound stigma-8(14)-en.3.beta.-ol (28.71%) which is the largest component in Miana leaf extract may also have insecticidal properties. This compound belongs to the stigmasterol derivative. Many researches have been done in examining the capacity of stigmasterol as insecticide. Stigmasterol has an insecticidal activities against rabbit ear mites *Psoroptes cuniculi* (Nong et al., 2017). Stigmast-7-en-3-ol is capable for killing lice *Myzus persicae* (Diaz et al., 2015).

The content of the active compound in Miana leaf extract propels the fast reaction of the extract in terminating houseflies. There is also a possibility that such fast reaction might be caused

by the diffusion of the Miana leaf extract sprayed directly into the test jar, where the flies are placed. The vapor of the Miana leaf extract is immediately absorbed by the insect skin, thus gives effects into the insects nervous system (Sulaiman & Kuswahyuningsih, 2008).

The *knockdown* process occurs when Miana leaf extract works by inhibiting a very important enzyme in the nervous system called cholinesterase (ChE). This enzyme becomes phosphorylated when bound to organophosphate, and this bond is permanent (irreversible). The effect of the inhibition of the ChE enzyme causes acetylcholine to accumulate at synapses, so that the houseflies experience muscle spasms and eventually become paralyzed or even die (Lee et al, 2017).

The results of this study indicates that the presence of chemical compounds from the methanolic extract of Miana leaf (*Coleus blumei*) plays an important role in the *knockdown* effect in houseflies (*Musca domestica*). This study provides information that Miana leaves can be used as an alternative in minimizing the use of botanical pesticides to control houseflies.

CONCLUSION

The results of phytochemical screening showed methanol extract of Miana leaf (*Coleus blumei*) containing chemical compounds alkaloids, tannins, flavonoids, saponins and terpenoids; while the GCMS analysis showed 7 chemical compounds identified in Miana leaf extract, namely (2, 4, 4, 4, 16, 16-D6) -3.alpha., 17.beta.-dihydroxy-5.beta.-androstane,(E,E)-3,7,11-trimethyl-2,6,10-dodecatrien-1-olacetate , 1,8-Bis(3,4-dicyanophenyl) anthracene, 23-R-methylcholesterol , Stigmasterol , Stigma-8(14)-en-3.beta.-ol, and alpha -Amyrin acetate. The results of the toxicity test showed that a concentration of 30% and 40% could result in *knockdown* effect that was accompanied by death with a percentage of 100%. It can be concluded that the methanolic extract of Miana leaves has the potential as an anti-housefly (*Musca domestica*).

Suggestion furthermore, further research is needed to test the ability of miana leaves to other insect species so that they can become promising alternative plants in the field of integrated pest management in Indonesia.

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REFERENCES

Cakir, A. (2004). Essential oil and fatty acid composition of the fruits of Hippophae rhamnoides L.(Sea Buckthorn) and Myrtus communis L. from Turkey. *Biochemical systematics and ecology*, 32(9), 809-816.

Chandrasekaran, M., Venkatesalu, V., Anantharaj, M., & Sivasankari, S. (2005). Antibacterial activity of fatty acid methyl esters of Ipomoea pescaprae (L.) Sweet. *Indian drugs*, 42(5), 275-281.

Chaudhary, J., Kaur, N., Jain, A., & Koshore, L. (2011). Stigmasterol: A Comprehensive Review. *International Journal Of Pharmaceutical Science And Research*, *2*(9), 2259-2265.

Chaveerach, A., Sudmoon, R., & Tance, T. (2017). Interdisciplinary researches for potential developments of drugs and natural products. *Asian Pasific Journal of Tropical Biomedicine*, *7*(4), 378-384.

Choi, J. M., Lee, E. O., Lee, H. J., Kim, K. H., Ahn, K. S., Shim, B. S., Song, M. C., Baek, N. I., & Kim, S. H. (2007). Identification of Campesterol from *Chrysanthemum coronarium* L. and its Antiangiogenic Activities. *Phytotherapy Research*, *21*, 954-959.

Cole, D. C., Carpio, F., & Leon, N. (2000). Economic burden of illness from pesticide poisonings in highland Ecuador. *Rev. Panam. Salud Publica 2000*, *8*, 196–201.

Damalas, C. A. & Eleftherohorinos, I. G. (2011). Pesticide exposure, safety issues, and risk assessment indicators. *International Journal Environment Res. Publ. Health, 8*, 1402–1419.

Darmadi & Anita, D. (2018). Uji Mortalitas Lalat Rumah (*Musca domestica*) Setelah Pemberian Ekstrak Kulit Duku (*Lansium domesticum* Corr.). *Jurnal Analis Kesehatan Klinikal Sains, 6*(1), 18-23.

De Stefani, E., Boffetta, P., Ronco, A. L., Brennan, P., Deneo, P. H., Carzoglio, J. C., & Mendilaharsu, M. (2000). Plant sterols and risk of stomach cancer a case-control study in Uruguay. *Nutr. Cancer.*, *37*, 140-144.

Elbermawy, S. M., Elkattan, N. A. I., Ahmed, K. S., & Gawad, A. R. M. (2011). Screening for bioactive plant materials against house fly, *Musca domestica* L. (Diptera: Muscidae). *Functional Plant Science and Biotechnology*, *5*(1), 45-51.

European Food Safety Authority (EFSA). (2012). Scientific Opinion on the safety of stigmasterol-rich plant sterols as food additive. *EFSA Journal*, *10*(5), 1-39.

Ferrer, A., Altabella, T., Arro, M., & Boronat, A. (2017). Emerging roles for conjugated sterols in plants. *Progress in Lipid Research*, *67*, 27-37.

Fitriana. (2014). *Uji Potensi Minyak Atsiri Daun Selasih Ungu (Ocimum sanctum) Sebagai Insektisida Nabati Terhadap Perkembangan Lalat Rumah (Musca domestica) Di Laboratorium* (Undergraduate thesis). Retrieved from UIN Sunan Kalijaga Yogyakarta Database.

Gaag, V. N. (2000). Pick your poison. *New Int.*, *58*,

9-11.

Hastutiek, P. & Fitri, L. E. (2007). Potensi *Musca domestica* Linn. Sebagai Vektor Beberapa Penyakit. *Jurnal Kedokteran Brawijaya*, *23*(3), 125-136.

Heras, D. B. L. & Hortelano, S. (2009). Molecular basic of the anti-inflammatory effect of the terpenoids. *Inflammation and Allerg- Drug Targets*, *8*, 28-39.

Hernandez, V. L., Mangas, S., Palazon, J., & Navaro-Ocana, A. (2010). Valuable medicinal plants and resins: Commercial phytochemicals with bioactive properties. *Ind. Crop. Prod.*, *31*, 476-480.

Ibrahim, A. M. A., Ahmed, H. H. S., Adam, R. A., Ahmed, A., & Elaagip, A. (2018). Detection of Intestinal Parasites Transmitted Mechanically by Houseflies (*Musca domestica*, Diptera: Muscidae) Infesting Slaughterhouses in Khartoum State, Sudan. *International Journal of Tropical Disease, 1*(1)*,* 1-5.

Kangsamaksin, T., Chaihongyot, S., Wootthichairangsan, C., Hanchaina, R., Tangshewinsirikul, C. & Svasti, J. (2017). Lupeol and Stigmasterol suppress tumor angiogenesis and inhibit cholangiocarcinoma growth in mice via downregulation of tumor necrosis factor. *Plos One*, *12*(12), 1-16.

Kardinan, A. (2007). Daya Tolak Ekstrak Tanaman Rosemary (*Rosmarinus officinalis*) Terhadap Lalat (*Musca domestica*). *Bul. Littro*, *18*(2), 170-176.

Kumalasari, E., Setyawati, T. R. & Yanti, A. H. (2015). Daya Tolak Ekstrak Metanol Daun Kesum (*Polygonum minus* Huds.) Terhadap Lalat Rumah (*Musca domestica* L.). *Protobiont, 4*(2), 40-47.

Lee, C. Y., Dang, K., Dogget, S. L. & Singham, G. V. (2017). Review: Insecticide resistance and resistance mechanisms in bed bugs, *Cimex spp.* (Hemiptera: Cimicidae). *Parasite & Vectors, 10*(318), 1-31.

Lide, D. R. (2005). *CRC Handbook of Chemistry and Physics. Boca Raton: CRC Press. ISBN 0-8493-0486-5.*

Mahatheeranont, S., Suttiarporn, P., Chumpolsri, W., Luangkamin, S., Teepsawang, S. & Learskamolkarn, V. (2015). Structure of Phytosterols and Triterpenoids with Potential Anti-Cancer Activity in Bran of Black Non-Glutinous Rice. *Nutrients*, *7*, 1672-1687.

McCann, S. E., Freudenheim, J. L., Marshall, J. R., & Graham, S. (2003). Risk of human ovarian cancer is related to dietary intake of selected nutrients, phytochemicals and food groups. *The Journal of nutrition*, 133(6), 1937-1942.

Mondul, A. M., Rohrmann, S., Menke, A., Feinleb, M., Nelson, W. G., Platz, E. A., & Albanes, D. (2011). Association of Serum α-Tocopherol with Sex Steroid Hormones and Interactions with Smoking: Implications for Prostate Cancer Risk. *Cancer Causes Control, 22*(6), 827-836.

Moreira, M. D., Picanco, M. C., Barbosa, L. C. A., Guedes, R. N. C., Campos, M. R., Silva, G. A., & Martins, J. C. (2007). Plant compounds insecticide activity against Coleoptera pests of stored products. *Pesq. Agropec. bras, 42*(7), 909-915.

Nong, X., Yang, Y.J., Yang, G.Y., Chen, F.Z., Tang, M. & Wang, G. (2017). Toxicity of Stigmasterol Isolated from Crofton Weed, *Eupatorium adenophorum* Spreng. Against a Rabbit Ear Mite, *Psoroptes cuniculi*. *Pakistan J. Zool., 49*(4), 1197-1200.

Palazon, J., Vasquez, L. H.*,* & Navaro-Ocana, A. (2012). The The Pentacyclic Triterpenes α, β-amyrins: A Review of Sources and Biological Activities. *Phytochemicals, 23*, 487-502.

Purwita, A., Indah, K. N., & Trimulyono, G. (2013). Penggunaan Ekstrak Daun Srikaya (*Anonna squamosa*) Sebagai Pengendali Jamur *Fusarium oxysporum* Secara *In Vitro*. *Jurnal Unesa, 2*(2), 179-183.

Rizvi, S., Raza, S. T., Ahmed, F., Ahmad, A. Abbas, S., & Mahdi, F. (2013). The Role of Vitamin E in Human Health and Some Disease. *Sultan Qaboos University Medical Journal, 14*(2), 157-165.

Selvamangai, G. & Bhaskar, A. (2013). Analysis of phytocomponents in the methanolic extract of *Eupatorium triplinerve* by GC-MS method. *International Journal of Drug Development & Research, 5*(1), 384-391.

Singh, A. & Kaur, J. (2016). Toxicity of Leaf Extract of *Ricinus communis* L. (Euphorbiaceae) Against the Third Instar Larvae of *Musca domestica* L. (Diptera: Muscidae). *American Journal of Bioscience, 4(3-1),* 5-10.

Soares, W. L. & Porto. M. F. D. (2009). Estimating the social cost of pesticide use: An assessment from acute poisoning in Brazil. *Ecol. Econ., 68,* 2721–2728.

Sulaiman, T. N. S. & Kuswahyuningsih. (2008). Teknologi Farmasi dan Formulasi Sediaan Semi Padat. UGM: Yogyakarta.

Sumita, Y., Kawada, H., & Minakawa, N. (2016). Mode of entry of a vaporized pyrethroid knockdown agent into the body of the housefly, *Musca domestica* (Diptera: Muscidae). *Appl. Entomol. Zool.*, *51*, 653-659.

Traber, M. & Manor, D. (2012). Vitamin E. *American Society for Nutrition Adv. Nutr.*, *3*, 330-331.

Wijayakusuma, H. (2005). Menumpas penyakit kewanitaan dengan tanaman obat. Jakarta: Puspa Swara.

Wirawan, I. A. (2006). *Insektisida Pemukiman dalam Hama Pemukiman Indonesia. Pengenalan, Biologi dan Pengendalian Unit Kajian Pengendalian Hama Pemukiman (UKPHP)*. Bogor: Bogor: Fakultas Kedokteran Hewan IPB.

Yuliana, V., Yamtana, & Kadarusno, A. H. (2016). Aplikasi Penyemprotan Perasan Daun Kamboja (*Plumeria acuminate*) Terhadap Kematian Lalat Rumah (*Musca domestica*). *Jurnal Kesehatan Lingkungan*, *13*(1), 299-306.

Yuliani, S., Sri, U., & Nanan, N. (2005). Efektivitas Lilin Penolak Lalat (Repelen) dengan Bahan Aktif Limbah Penyulingan Minyak Nilam. *Jurnal Pascapanen*, *2*(1), 1-10.