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Identification and toxicity test of *Orthosiphon stamineus* leaves extract against Houseflies (*Musca domestica*)

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ABSTRACT

Houseflies (*Musca domestica*) are generally controlled using chemical pesticides that leave residues, which are harmful to health and environment. Pests become resistant in the process. To overcome this, it is necessary to use plant-based pesticides from natural ingredients. This research suggests the use of the leaves of cat's whiskers (*Orthosiphon stamineus*). Cat's whiskers leaves are known to have pharmacological activities such as diuretic drugs, fever-reducing drugs, diabetes drugs, anti-inflammatory and antibacterial agents. The content of chemical compounds found in the leaves of cat's whiskers is considered to play a role in these activities. The purpose of this study is to determine the content of chemical compounds (alkaloids, tannins, flavonoids, saponins, and essential oils) in the leaves of cat's whiskers and their toxicity to houseflies (*Musca domestica*) with concentrations 10%, 20%, 30% and 40%. The extracts were observed for 5 and 15 minutes. The cat whiskers leaves were extracted through maceration, using n-hexane solvent for 3 days. The n-hexane extract of cat's whiskers leaves tested through phytochemicals process using chemical reagents. Their toxicity towards was then tested against houseflies. The phytochemical screening of the n-hexane cat whiskers shows the presence of alkaloid compounds and saponins.The toxicity test shows that the 40% concentration was the most effective in killing flies (100% mortality percentage) within 5 minutes. It can be concluded that the extract of cat's whiskers has the potential to be anti-houseflies.

Key words: *Orthosiphon stamineus, Chemical compounds, Musca domestica, Mortality*

Introduction

Among thousands of insect species, houseflies (*Musca domestica*) is the most common insect in the world (Sinthusiri *et al.*, 2013). Houseflies are perceived to be dirty animals (unhygienic) and their existence should be watched out for. Houseflies are multiplying on the media in the form of feces, meat, garbage, animal waste and waste that contain many disease agents (such as viruses, bacteria, protozoa, worms, amoeba and others). Thus they are easily contaminated by good disease agents in the stomach and in the mouth and feet. Houseflies are called mechanical vectors or disease-carrying intermediaries. They carry the disease agents which are transmitted to humans/animals when houseflies land on food. The habit of flying, then going back and returning from feces to food is the plausible process of disease transmission (Sigit *et al.*, 2006).

At present, techniques for controlling houseflies still rely on the use of chemical insecticides. This method is considered to be unfavourable because

the use of chemical insecticides are generally toxic. Pest is resistant to pesticides. The negative effects on non-target organisms, cannot be decomposed. They leave residues in the environment and enter the food chain (Sripongpun, 2008; Sinthusiri *et al.,* 2013).

To reduce environmental pollution, various types of plants are currently being developed as plant-based pesticides. In Indonesia, there are many types of plants and germplasm that produce secondary metabolites that can be used as plant-based pesticides, such as the roots, stems, leaves, flowers, fruits and seeds. According to Selem and El-Sheikh (2015), plant extracts are not only used as medicinal ingredients, but they also have potentials as plant-based pesticides. Most of the plant extracts are insecticidal against houseflies (*Musca domestica*) (Sukontason *et al.,* 2004; Cakir *et al.,* 2008). One of the plants that can be used as plant-based pesticides is the cat's whiskers plant (*Orthosiphon stamineus*).

According to Khater (2012), chemical compounds produced from the Lamiaceae family plant have great potentials as an insecticide. The results of research conducted by Ningsih *et al.* (2016), shows that the ethanol extract 96% of cat's whiskers leaves (*Orthosiphon aristatus*) function as insecticides against brown plant hopper pests (*Nilaparta lugens*).

From the above background, this study is carried out to determine the chemical content of cat whiskers leaves extracted with n-hexane solvent using maceration method and its potential to control houseflies (*Musca domestica*).

Materials and Methods

Sample Preparation

Cat's whiskers (*Orthosiphon stamineus*) were collected from Juanda Sidoarjo, East Java, Indonesia (Figure 1). The leaves were picked, washed and then dried. Then leaves dried samples of the cat's whiskers were chopped, blended, and sieved until they became fine powder.

Sample Extraction

A total of 30 g of fine powder of cat's whiskers leaves was macerated (soaked) into a glass jar containing 600 mL of n-hexane solvent. The maceration process was conducted for 3 days. The mixture was stirred occasionally. After 3 days, the mixture was filtered with filter paper. The similar process was repeated twice.

Fig. 1. *Orthosiphon stamineus*

Phytochemical Testing

A total of 100 mL of phytate was used for the phytochemical testing process using chemical reagents. Phytochemical testing method was carried out, following the research conducted by Salve and Kakde (2018).

1. Alkaloid Test

a. Dragendorff Test
A total of 5 mL of the extract was added with 4 drops of Dragendorff reagent. If a reddish orange sediment is formed, it showed a positive presence of alkaloids.

b. Wagner test
A total of 5 mL of test extract was added with 4 drops of Wagner reagent. When a reddish brown sediment was formed, it showed a positive presence of alkaloids.

c. Mayer test
A total of 5 mL of the test extract was added with 4 drops of Mayer reagent. If a yellowish white sediment was formed, it indicated an alkaloid.

2. Flavonoid Test

A total of 5 mL extract sample was added with 1 mL Pb acetate solution. Positive the presence of flavonoids when yellow deposits form.

3. Saponin Test

A total of 5 mL of extract sample was added into a test tube, then mixed with 5 mL of hot distilled water. When the foam was, there was the positive saponin.

4. Tanin Test

A total of 5 mL of the extract sample was added into a test tube, and then mixed with 5 drops of FeCl3 solution. When there was dark blue or greenish blanck change, it indicated the positive existence of tannin.

5. Essential Oil Test

A total of 3 mL of the extract sample was evaporated in a porcelain cup. If there was the distinctive odor, this indicates the positive results of essential oil.

Bioassay Testing towards Houseflies (*Musca domestica*)

1. Making Spray
Cat's whiskers leaves extract spray is made using several concentration series, namely 5%, 10%, 20%, 30% and 40%. Concentration of 5%, obtained by dissolving 5 mL of n-hexane extract of cat's whiskers leaves into a 100 mL sprayer bottle containing 95 mL of distilled water.

2. Extract Toxicity Test
The testing process of the effectiveness of the repellent is done in accordance to method of Kardinan (2007) with some modifications to the tool and exposure time.

The top of the test jar was perforated for air circulation and also as a medium for entering the test extract by spraying (spray method). Twenty-five houseflies were put into test jars that had been labeled according to the determined concentrations. The mixture was sprayed from a distance of ± 5 cm extract of cat's whiskers leaves using a spray bottle directly aimed at the flies in the jar with a dose of 2 times the spray (@ 0.5 mL).

Observations were made by counting the number of flies that fell and died every 5 minutes, 10 minutes and 15 minutes.

3. Calculation of Mortality Percentage of Houseflies

Percentage mortality of houseflies using the formula according to Kardinan (2007) as follows:

$$\text{Mortality (\%)} = \frac{\text{the number of dead flies}}{\text{number of initial flies}} \times 100$$

Results and Discussion

Phytochemical Screening

Phytochemical screening was carried out as a preliminary qualitative test to find out secondary metabolite compounds contained in cat's whiskers extract, such as alkaloids, tannins, flavonoids, alkaloids, terpenoids and essential oils. Phytochemical screening results of n-hexane extract of cat's whiskers are presented in Table 1.

The results in Table 1 show that the secondary metabolite compounds extracted from cat whiskers leaves with n-hexane contain alkaloids, saponins and essential oils.

Toxicity Test of Cat's Whisker Leaves Extract Against Houseflies (*Musca domestica*)

The toxicity of cat's whiskers extract to the domestic flies can be seen from the flies mortality which is calculated for 5 minutes, 10 minutes and 15 minutes. The parameters indicate the fly *knockdown* that is permanent. It is followed by death or only *knockdown* recovery. Data on mortality of the houseflies can be seen in Table 2.

After being exposed to exposure for 15 minutes

Table 1. Phytochemical Screening Results of n-hexane Extract of Cat's Whiskers Leaves

Compound Test	Methods	Results	Observations
Alkaloid	Mayer Reagents	+	White sediment formed
	Wagner's Reagent	+	Brown sediment formed
	Dragendorff Reagents	+	Orange sediment formed
Tannin	$FeCl_3$	-	Blackish green color is not formed
Flavonoid	Pb asetat	-	Yellow color is not formed
Saponin	Aquadest	+	Embossed foam for 1 minute
Essential Oil	Distinctive odor	+	Special odor is formed

Table 2. Houseflies Mortality

Exposure Time (minute)	Mortality (%)			
	10%	20%	30%	40%
5	40	68	80	100
10	80	92	100	100
15	100	100	100	100

by extracting cat's whiskers leaves at various concentrations that were sprayed, the results indicate that all concentrations of cat's whiskers leaves had a significant mortality effect, especially at a concentration of 40%.

Table 2 shows that the concentration of extractive substances given is directly proportional to the mortality of houseflies. This is because the extractive substances in large concentrations have more extractive substances, so they are more toxic thanthe substances with small concentrations.

Treatment of n-hexane extract of cat's whiskers leaves with a concentration of 10%, 20%, 30% and 40% can cause mortality in *M. domestica*, but there is a difference in the time to give the first *knockdown* effect. Concentration of 10% takes longer to kill all the flies in fifteen minutes. Concentration of 40% is the most rapid concentration causing mortality of large population of houseflies in five minutes (Table 2). This is in accordance with the research conducted by Memmi (2010) which states that the time of *knockdown* (which is followed by death) in houseflies is increasingly occurring along with the increasing dose of insecticide given. Likewise in the study of Kardinan (2007), which showed that the domestic flies' mortality increased at a concentration of 20% within 30 minutes.

The high mortality rate of houseflies is thought to be caused by the insecticidal effect of secondary metabolite compounds dissolved in n-hexane. This is because the n-hexane extract of cat's whiskers leaves contain alkaloids, saponins and essential oils. According to Riaz *et al.* (2018), these compounds are insecticidal.

Based on the method of exposure to n-hexane extract of cat's whiskers leaves using the spray method, the houseflies poisoning is suspected to happen through the respiratory system and nervous system. According to Kostyukovsky *et al.* (2002), symptoms of disruption of the nervous system in insects include uncontrolled movements and tremors (muscle spasms) followed by *knockdowns* (falls). Secondary metabolites of alkaloids, terpenoids, saponins, phenols, flavonoids and essential oils can disrupt the respiratory system and nervous system in insects.

Alkaloid compounds act as the nerve poisons which inhibit the action of the acetylcholinesterase enzyme. The acetylcholinesterase enzyme is a very important enzyme in the nervous system of insects. The inhibition of the action of the acetylcholinesterase enzyme causes the accumulation of acetylcholine, causing chaos in the impulse delivery system to the muscular system. This condition results in muscle spasms, *knockdown* and causes death (Sanjaya and Tina, 2006).

Saponin compounds, including compounds that are toxic to insects. Saponin compounds damage cell membranes and disrupt the metabolic processes of insects (Pitojo *et al.,* 2003; Heras and Hortelano, 2009).

Essential oils produced by plants have insecticidal properties, namely as an antifeedant and prevent oviposition of various insect pests including houseflies (Isman, 2006; Koul *et al.,* 2008). Essential oil compounds have a distinctive odor or aroma. Essential oils including phenolic compounds have irritative properties on the skin. They cause a burning sensation in insects. In addition this compound also inhibits the work of the sense of smell of flies (of factory sensory), so that the fly does not recognize its food and will go away (is repellent) (Manaf *et al.,* 2012).

Conclusion

Based on the research, the following conclusions are obtained:

1. Secondary metabolite compounds contained n-hexane extract of cat's whiskers are alkaloids, saponins and essential oils.
2. Cat's whiskers (*Orthosiphon stamineus*) leaves extract has the power of poison against houseflies (*Musca domestica*) which has the potential to be developed as a vegetable insecticide.
3. The most effective concentration of cat's whiskers leaves extract in killing houseflies is a concentration of 40% with a mortality percentage value of 100% at the 5th minute.

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