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Submission date: 16-Dec-2020 08:00PM (UTC+0700)
Submission ID: 1476707823
File name: SURAHMAIDA_Jurnal_Internasional_IJSR.pdf (930.01K)
Word count: 2867
Character count: 16473

International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Ocimum sanctum: Phytochemical Screening and its Activity as Biopesticide against *Musca domestica*

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Abstract: ***A study of basil leaf extract (Ocimum sanctum) phytochemical screening and its potential as a botanical pesticides against houseflies (Musca domestica) has been carried out. The research method used was extracting basil leaves with maceration method using n-hexane solvents, phytochemical screening using chemical reagents and GCMS and its toxicity to houseflies by spray method. Variations in extract concentrations were 5%, 10%, 20%, 30% and 40% and counted the number of dead flies at intervals of 5 minutes, 10 minutes and 15 minutes. The results of phytochemical screening with chemical reagents showed the presence of alkaloids, flavonoids and essential oils; and chemical compounds identified by GCMS, among others neral; 2,4- (D2) menth-2-ene; 4,5-Diclorotricyclo [5.3.1.1 (2,6)] dodec-3-en-11-one; 1- (4-phenylcyclohexyl) -1-hexanone; ethyl linoleolate; 3 (5) - (4'-Chlorophenyl) -4-nitroso-5 (3) -phenylaminopyrazole; eicosane; 2- (Acetoxymethyl) -3- (methoxycarbonyl) biphenylene; 6H, 16H, 31H-5,9: 15,19-Dimethano-10,14-metheno-26,30-nitrilo-5H, 25H-dibenzo [b, s]. The toxicity test results showed that at a concentration of 10% -40%, n-hexane extract was able to kill Musca domestica by 100% in the first 5 minutes of observation. It can be concluded that basil leaf extract can control houseflies.***

Keywords: Bioactive compounds of n-hexane extract of *Ocimum sanctum* leaves, houseflies, mortality

1. Introduction

Musca domestica is a cosmopolitan type of insect, which can be found in all environments. Housefly habitats are generally in dirty environments, such as garbage, feces, household waste and other sources of waste that contain many pathogenic organisms that cause disease so that these insects can transmit disease agents from one animal to another or to humans [1]. Commonly caused diseases include typhus, cholera, dysentery, anthrax, hepatitis, intestinal worms, and leprosy [2].

So far the efforts made by the community to control houseflies are with synthetic pesticides, because they are more effective, easily available and practical [3]. However, synthetic pesticides cause insects to become resistant, resurgence of target insects and leave residues that pollute the environment, food and are harmful to health [4].

The use of botanical insecticides can be an effective and environmentally friendly alternative to pest control [5]. Various types of plants are known to have potential as botanical pesticides because of the content of bioactive compounds in them, such as alkaloids, saponins, tannins, flavonoids and terpenoids. Indonesia has various types of plants that have the potential as biopesticides, one of which is basil (*Ocimum sanctum*).

Basil (*Ocimum sanctum*) is one of the members of the Lamiaceae family whose leaves are often consumed as fresh vegetables, flavorings and used as medicinal plants. This plant is easily found and can grow anywhere. According to [6], the bioactive compounds of *Ocimum* plants act as insecticides, fungicides, nematicides and also as antibacterials. In addition, the aroma of essential oils extracted from basil leaves and flowers is used for perfumes, pharmaceutical preparations and food additives.
Research studies that have been carried out on the utilization of basil plants as a botanical biopesticide include *Ocimum sanctum* essential oil capable of killing *M. domestica* larvae by 52% at a concentration of 20% [7], *Ocimum basilicum* essential oil at a concentration of 10% has the potential to repellent *Anopheles gambiae* mosquitoes [8], lotion from essential oil of *Ocimum basilicum* L. has a repellent effect on *Aedes aegepty* L. [9], concentration of 20% of *Ocimum basilicum* leaves oil can kill housefly larvae (*Musca domestica*) at 83% [10] and *Ocimum suave* essential oil is toxic to houseflies (*Musca domestica*).

Based on the description above, there is not yet research that utilizes basil leaves (*Ocimum sanctum*) as a botanical pesticide. Therefore this study aims to determine the presence of bioactive compounds found in n-hexane extract of basil leaves and their potential as a *Musca domestica* botanical pesticide in various concentrations.

2. Materials and Methods

2.1. Materials

The materials used in this study were basil leaves (*Ocimum sanctum*), houseflies (*Musca domestica*), n-hexane solvents, Mayer reagents, Wagner reagents, $FeCl_3$ solution, aquades, 2% NaOH solution, concentrated H_2SO_4 solution, chloroform, filter paper. The tools used include jars, erlenmeyer, funnels, pipettes, spray bottles, blenders, test tubes and GCMS.

2.2. Sample Preparation

Ocimum sanctum (Figure 1) was obtained from the area of Wonoayu, Sidoarjo, Indonesia. Then the basil was determined at the Biology Service Unit Airlangga University Surabaya to ensure that these plants were plant species that would be used in this study.

Figure 1: *Ocimum sanctum*

Samples of *Ocimum sanctum* leaves are washed to remove the sticky dirt and then dried. After drying, the dried sample leaves are mashed with a blender until it becomes a fine powder, then sieved with a filter. 30 grams of fine powdered basil leaves extracted by maceration method using 600 ml of n-hexane solvent for 3 days. During the maceration process, stirring is carried out every day to avoid solidifying the sample. After 3 days, filtration was carried out and obtained basil n-hexane extract. This maceration process is repeated twice.

2.3. Phytochemical Screening

In this study, the phytochemical screening method with chemical reagents was carried out based on the [11] method to determine the content of bioactive alkaloids, flavonoids, tannins and saponins. While the GCMS analysis was conducted at the Pharmacy Service Unit of Airlangga University Surabaya.

2.4. Making Extract Concentration

Variations in concentration used are 5%, 10%, 20%, 30% and 40%. To obtain a concentration of 5%: 5 ml of n-hexane extract of *Ocimum sanctum* leaves and added 95 ml of sterile distilled water, then put in a 100 ml spray bottle and so for the next concentration.

2.5. Toxicity test for *Ocimum sanctum* extract

The toxicity test of *Ocimum sanctum* leaves extract on houseflies is based on the study of [12] and [13] with some modifications. Prepared test jar whose lid has been given a hole for air circulation so that the test fly does not run out of oxygen and as a medium for spraying *Ocimum sanctum* leaves extract spray. Each test jar is labeled a predetermined concentration and contains 25 houseflies (*Musca domestica*). The toxicity method is in the form of n-hexane extract spray which is sprayed into a test jar containing 25 houseflies (*Musca domestica*) and then observed and calculated the number of dead houseflies every 5, 10 and 15 minutes. Repeated 3 times.

2.6. Calculation of houseflies mortality

Calculation of the percentage of mortality according to [14] is as follows:

$$Mortality = \frac{M2}{M1} x\ 100\%$$

with: M1 : the initial amount of houseflies (25 flies)
M2 : number of dead houseflies

3. Results and Discussion

3.1. Phytochemical Screening

Qualitative analysis was carried out by phytochemical screening to determine the presence of alkaloid compounds, flavonoids, tannins and saponins. GCMS is a tool that is usually used to identify and analyze compounds in traditional medicines and medicinal plants. GCMS techniques are widely applied to analyze non-polar components, volatile volatile oils, fatty acids, fats and alkaloids [15]. The results of screening bioactive compounds in the *Ocimum sanctum* leaves n-hexane extract are presented in the following Tables 1 and 2:

Table 1: The results of phytochemical screening of *Ocimum sanctum* leaves n-hexane extract

Compound	*Result*
Alkaloid	+
Flavonoid	+
Tanin	-
Saponin	-
Essential oil	+

From Table 1 *Ocimum sanctum* leaves n-hexane extract shows the presence of alkaloids, flavonoids and essential oils. In Table 2, it shows 9 bioactive compounds identified using GCMS, including Neral; 2,4- (D2) menth-2-ene; 4,5-Dichlorotricyclo [5.3.1.1 (2,6)] dodec-3-en-11-one; 1- (4-phenylcyclohexyl) -1-hexanone; Ethyl linoleolate; 3 (5) -(4'-Chlorophenyl) -4-nitroso-5 (3) -phenylaminopyrazole; Eicosane; 2- (Acetoxymethyl) -3- (methoxycarbonyl) biphenylene; and 6H, 16H, 31H-5,9: 15, 19-Dimethano-10,14-metheno-26, 30-nitrilo-5H, 25H-dibenzo [b, s].

3.2. The toxicity test results of *Ocimum sanctum* leaves n-hexane extract on *Musca domestica*

Biopesticide activity of n-hexane extract of *Ocimum sanctum* leaves as an anti-housefly can be seen from the mortality of houseflies calculated at 5, 10 and 15 minutes after treatment. Mortality data for houseflies can be seen in Table 3.

Table 2: The results of the bioactive compounds of *Ocimum sanctum* leaves n-hexane extract were identified using GCMS

Peak	*RT*	*Name of Compound*
1	10.039	Neral
2	15.831	2,4-(D2)menth-2-ene
3	18.185	4,5-Dichlorotricyclo[5.3.1.1(2,6)] dodec-3-en-11-one
4	20.241	1-(4-phenylcyclohexyl)-1-hexanone
5	21.685	Ethyl linoleolate
6	27.279	3(5)-(4'-Chlorophenyl)-4-nitroso-5(3)-phenylamino pyrazole
7	28.525	Eicosane
8	28.685	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene
9	31.169	6H, 16H, 31H-5,9: 15, 19-Dimethano-10,14-metheno-26, 30-nitrilo-5H, 25H-dibenzo[b,s]

Table 3: Test results for toxicity of *Ocimum sanctum* leaves extract

Time (minute)	*Mortality (%)*				
	5 %	*10 %*	*20 %*	*30 %*	*40 %*
5	20	100	100	100	100
10	52	100	100	100	100
15	80	100	100	100	100

In Table 3 it can be seen that the *Ocimum sanctum* leaves extracted with n-hexane solvents have stronger toxic compounds because with 5% concentration it can cause the percentage of housefly mortality to reach 80%. At concentrations of 10% - 40%, it shows a large mortality of termites, which means that the greater the concentration given is directly proportional to the mortality of houseflies (marked by the death of a faster housefly). According to [16], high extract concentrations will work faster in suppressing the activity of the nervous system of insects so that insects experience *knockdown* and eventually die when compared with lower concentrations. This is in accordance with the research conducted by [12], where high concentrations of essential oils of *Rosmarinus officinalis* caused the death of high houseflies.

The mechanism of toxicity is after spraying *Ocimum sanctum* leaves n-hexane extract into a test jar containing houseflies, so the spray evaporates into the air. Then the insect will detect the presence of chemical stimuli (in the form of aromas) through the olfactory receptor organ and are passed on by the sensory nerve axons to the brain, so that insects will try to avoid the scent if it is considered dangerous and disliked or approached if deemed attractive. The high mortality value of houseflies is thought to be caused by the insecticidal effect of the chemical compound of n-hexane extract of *Ocimum sanctum* leaves, namely alkaloids, flavonoids, and essential oils. Chemical compounds identified with GCMS are components of essential oils.

These compounds enter to the body of houseflies through the respiratory system and affect the acetylcholin receptors in the nervous system [17]. These chemical compounds are toxic even at small concentrations. Chemical compounds such as alkaloids, flavonoids, terpenoids and essential oils can interfere with the respiratory system and nervous system [18]. Alkaloids, flavonoids and essential oils are toxic compounds that inhibit the action of the acetylcholinesterase enzyme. The acetylcholinesterase enzyme is the most important enzyme in insects and mammals [19]. Acetylcholine acts as a deliver of impulses from nerve cells to muscle cells through the synapse. After the impulse is delivered, acetylcholine will convert acetylcholine to acetic acid and choline, so that the synapse becomes empty and acetylcholine can deliver the next impulse. However, because the acetylcholinesterase enzyme is inhibited, acetylcholine accumulation results in chaos in the impulse delivery system to the muscular system. As a result insects experience muscle spasms and paralysis [20]-[23]. In addition, flavonoids interfere with the insect's respiratory system which causes death in insects because they cannot breathe [24]. Essential oils and their components are plant natural substances, including terpenoid compounds, are neurotoxic compounds and are generally used as insect repellents. The repellent mechanism of essential oils is by inhibiting the activity of insect acetylcholinesterase [19].

Overall, this study shows that the biopesticide activity of *Ocimum sanctum* leaves extract can be seen from the mortality of houseflies (*Musca domestica*). The chemical compounds of the *Ocimum sanctum* leaves play an important role in the mortality of houseflies. It can be concluded that *Ocimum sanctum* leaves not only can be used as medicinal plants, but also have the potential as an alternative plant to substitute synthetic pesticides in controlling insects (ie houseflies).

4. Conclusion

1) The n-hexane extract of *Ocimum sanctum* leaves has a toxic effect on house flies and has the potential as a botanical biopesticide
2) *Ocimum sanctum* leaves extract has a strong toxicity because at a concentration of 5% it can kill house flies by 80%, and at a concentration of 10% - 40% produces the highest mortality of houseflies which is equal to 100%
3) The chemical compounds contained in *Ocimum sanctum* leaves of n-hexane extract play an important role as insecticides

5. Acknowledgements

The author would like to thank DRPM Ristekdikti for funding this research through the Beginner Research Lecturer in 2019.

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