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RESEARCH ARTICLE

Efficacy of plant extracts against the immature stage of house fly, *Musca domestica* (Diptera: Muscidae)

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ABSTRACT

This research aimed to find indigenous plants and suitable solvents to extract substances with the capacity to suppress the immature stages of house fly populations in animal farms and urban areas. Seven native Thai plants were tested: *Alstonia scholaris* (L.) R.Br., *Murraya paniculata* (L.) Jack, *Citrus aurantium* L., *Colocasia esculenta* (L.) Schott, *Limnophila aromatica* (Lam.) Merr., *Persicaria odorata* (Lour.), and *Manihot esculenta* Crantz. Solvents with different polarities were used in series (hexane, ethyl acetate, acetone, ethanol, and water) to extract the active compounds from the plant tissues. The effects of extracts on immature stage were assessed separately *in vitro* using a completely randomized design with 5 replicates. The effects of each plant extract on the house fly stages varied depending on the solvent utilized. Extracts with high polarity solvents (ethanol and acetone) showed strong ovicidal activity but for larval and pupal stages, hexane, a low polarity solvent, demonstrated significant larvicidal and pupicidal activity. Acetone and ethanol solvents of *P. odorata* and *L. aromatica* caused notable mortality rate for the egg stage. Hexane extracts of *M. paniculata* and both hexane and ethyl acetate extracts of *C. aurantium* induced the highest percentage of larval mortality. Even if other plant extracts have less of an impact on the mortality of house fly eggs or larvae, they have an impact on the growth and development. The results showed that most plant extracts based on various solvents caused considerable mortality in house fly pupa. In this study, the hatching percentage of adult females was lower than the adult males after specific plant extracts were applied throughout the larval and pupal phases. High-efficiency plant extracts' LC_{50} and LC_{90} values for house fly immature stages were calculated. The acetone extract of *P. odorata* during the egg stage had LC_{50} and LC_{90} values of 7.816 and 31.117 mg/mL, respectively. At the larval stage, *M. paniculata*'s hexane extract had concentrations of 4.865 and 22.284 mg/mL, while *C. aurantium*'s ethly acetate extract had concentrations of 26.424 and 61.801 mg/mL. Significant active chemicals discovered by GC-MS analysis were included bioactive substances with insecticidal properties, including flavonoids, alkanes, coumarins, etc., were identified by GC-MS analysis.

Keywords: Biopesticide; solvent; bioactive compound.

INTRODUCTION

House flies (*Musca domestica* Linnaeus, 1758) are major medical and veterinary pests. They are one of disease-carrying pests that affect people and animals, transmitting pathogenic microorganisms such as helminthic eggs, protozoa cysts and trophozoites, bacteria, fungi, and viruses (Issa, 2019). Additionally, house flies can cause irritation and annoyance to people and animals during outbreaks. According to Geden *et al*. (2021), house fly damage may cause losses of up to $1 billion annually in the US. because it transmitted diseases to humans and animal such as typhoid fever, cholera, tuberculosis, and salmonellosis (Graczyk *et al*., 2005). A female housefly can lay 900 eggs in a lifetime (West, 1951) in multiple batches of 100 to 150 eggs (Geden *et al*., 2021). Due to its high reproductive capacity, house fly control is necessary in urban areas and animal farms. Because controlling immature stages is essential to controlling adult populations, breeding sites and immature habitats such as organic waste and garbage are the primary focus of house fly management (Hinkle & Hogsette, 2021).

Chemical control has been the most often utilized method in house fly management programs because it is affordable, quick-acting, available to purchase locally, and effectively controls the target pests. (Cooper & Dobson, 2007; Mahr *et al*., 2008; Aktar *et al*., 2009). However, Overuse of chemicals has resulted in toxic residues persisting in the environment, risks to other organisms, and the development of resistance to synthetic chemicals (Aktar *et al*., 2009; Denholm & Devine, 2013). For the purpose of controlling house fly larvae, insecticidal formulations can be added to animal feed or sprayed directly into contaminated breeding substrate. Animals may receive formulations as treatments to their feed. When the active

component of the insecticide passes through the digestive system, it releases insecticidal residues in the excrement (Moon, 2002).

Insect control methods have recently shifted their focus to bio-pesticides made from plant extracts due to the ecological concerns associated with chemical insecticides. This is because biodegradable, ecologically friendly, and less hazardous than chemical insecticides (Kubinyi, 2002; Godlewska, 2020). Plant extracts have been reported as insect repellents, growth inhibitors, anti-feeding, and oviposition-deterrents (Koul, 2005). There have been reports that some plant extracts have the potential to suppress house flies. For instance, *Piper betles* L. has a contact toxicity to adult house flies (Anisah & Sukesi, 2018), *Moringa oleifera*, *Allium sativum*, and *P. nigrum* suppressed house fly development at all stages when extracts were administered as larval food during the larval stage (Nisar *et al.*, 2021). Attaullah *et al.* (2020) reported that *Azadirachta indica*, *Penganum harmal*, *Datura stramonium*, *Tribulus terrestris*, and *Chenopodium murale* caused inhibition of enzyme activity and growth regulators when applied to the larval stage.

Thailand is an ecologically diverse country, blessed with high biodiversity that includes a flora of vascular plants that surpasses 11,000 species (Panyadee *et al*., 2023). Some of them have bioactive ingredients with potential for traditional medicine and as pesticides (Nxumalo *et al*., 2021).

Limnophila aromatica and *P. odorata* are native plants throughout Southeast Asia. In Thailand, both of them are used as food ingredients. They have a strong smell, and there have been reports of insect toxicity. *L. aromatica* has been reported to have a high phenolic and flavonoid content (Kumar *et al.*, 2019), whereas *P. odorata* has been reported to have high levels of flavonoids, anthraquinone, coumarins, and steroids (Saleh Al-Faqeeh *et al*., 2020).

Murraya paniculata is a small tropical evergreen tree growing in Thailand. Due to its high content of volatile chemicals, it has several applications in traditional medicine. There have been reports of both nematicidal and antifungal activities (Dosoky *et al*., 2016). *Citrus aurantium* is an evergreen tree native to Southeast Asia. Its fruit is utilized because it is a source of flavonoid-type compounds with diverse biological effects (Suntar *et al.*, 2018). Even though essential oils and bioactive compounds have been reported from its leaves (Oulebsir *et al.*, 2022), few reported studies have focused on them. *Alstonia scholaris* and *Co. esculenta* were chosen since they are both native to the area and do not sustain pest damage. Both plants have significant phenolic content in their leaves (Itam *et al.*, 2018; Nur-Hadirah *et al.*, 2021). For *Ma. esculenta*, leaves are one of the agricultural wastes that remains after pruning and after harvesting the tubers. The leaves of *Ma. esculenta* contain phenolic compounds such as cyanidine, tannins, delphinidine, and anthocyanidins, which are recognized for having pharmacological activities (Taupik *et al.*, 2023).

According to Raja *et al.* (2014), each plant consists of various bioactive chemicals that have specific properties against distinct stages of insect life. Different compounds were extracted by different polarity solvents. Abdullahi and Haque (2020) and Pinelo *et al.* (2004) reported that the solvent was the main factor in the extraction of biochemical compounds from plants. Different bioactive substances have varying effects on the physiological targets of insects. In order to manage house flies at different phases of the extraction process, the solvent is crucial. While most studies concentrate on the larval stage, all stages of the life cycle are present at the breeding site: eggs, larvae, and pupae. Therefore, the main purpose of this research was to find the local plants and suitable solvents to extract substances with potential for control of immature stages of house fly in order to apply an environmentally friendly method of house fly control on animal farms and urban areas.

MATERIALS AND METHODS

Musca domestica and rearing

House fly colonies were maintained in the Department of Entomology, Khon Kaen University at room temperature (28±2°C), and relative humidity of 60±10%. Adults were reared in a mesh cage containing adult food, which was a 1:3 ratio of sugar to powdered milk (Tangkawanit *et al.*, 2018). Moist tissue paper and fish meal were provided in a petri-dish for adult oviposition. After house fly oviposition, egg clusters were transferred to a larval food container (18×27×12 cm) containing a mixture of 62.5 mL of water, 25 g of rice bran, and 12.5 g of chicken feed (Ardburi & Tangkawanit, 2022). After 5 days, larvae and food were transferred to a sieve box. The third instar larva passed through the sieve and developed into pupae in the dry box below. The pupae were then transferred to a different cage for emergence of adults. The house flies studied were from at least a 2nd generation colony.

Plant collection

Plants were chosen for screening based on factors such as plant waste (Plant-derived wastes from agriculture and processing), not damaged by insects (Castillo *et al.*, 2009), strong smells, bitterness, or astringency. Some of the active substances involved are based on phenols, flavonoids, isoflavones, terpenes, and glucosinolates, and there have been prior reports of their toxicity against insects (Akbar *et al*., 2022). Based on these criteria, seven plants occurring in Thailand were selected for testing of their biopesticide activity against house fly. These plants were: *Alstonia scholaris* (L.) R.Br., *Murraya paniculata* (L.) Jack, *Citrus aurantium* L., *Colocasia esculenta* (L.) Schott, *Limnophila aromatica* (Lam.) Merr., *Persicaria odorata* (Lour.), and *Manihot esculenta* Crantz. Seven local plants were selected (Table 1). For screening, different parts of each plant were collected (leaves and stems) depending on the reports of the toxicity of those plant parts.

Extraction process

Seven plants were extracted with solvents having different polarities: hexane, ethyl acetate, acetone, ethanol, and water (Pandey & Tripathi, 2014). Every plant part that was chosen (Table 1) was cleaned and weighed one kilogram., cut into small pieces, and dried under sunlight (33°C±2°C) for 3–5 days and then

Table 1. List of plant extracts used in this study

Scientific name	Family	Common name	Part
Alstonia scholaris (L.) R. Br.	Apocynaceae	Devil tree	Leaves
Murraya paniculata (L.) Jack.	Rutaceae	Orange jasmine	Leaves
Citrus aurantium L.	Rutaceae	Bitter orange	Leaves
Colocasia esculenta (L.) Schott	Araceae	Elephant ear	Leaves
Limnophila aromatica (Lam.) Merr.	Plantaginaceae	Rice paddy herb	Arial part
Persicaria odorata (Lour.)	Polygonaceae	Vietnamese coriander	Arial part
Manihot esculenta Crantz	Euphorbiaceae	Cassava	Leaves

ground to fine powder. Plant powders were soaked in hexane (1:3 liters). After 72 hours, plant material was filtered through filter paper (Whatman No. 1). The solvent was transferred to a rotary evaporator for concentration.

The remaining residue from hexane (98%) extraction was extracted with ethyl acetate (98%), acetone (99.8%), ethanol (95%), and water, respectively. Extraction processes has been provided in the methodology: the filtrates of hexane crude extract were concentrated using a vacuum rotary evaporator (Buchi Rotavapor R-114) under reduced pressure at 40°C, while the aqueous crude extracts were evaporated to dryness using a refrigerated bath. The residue obtained from each plant extract was left to cool at room temperature to remove traces of solvent, and then finally, it was collected separately in an amber glass bottle and preserved and refrigerated at 4°C until used for experimentation (Soonwera & Phasomkusolsil, 2015).

Bioassay

The experiments with different solvents were separately examined in the laboratory employing a completely randomized design (CRD) with 37 treatments and 5 replications. Each plant extract was prepared from a stock solution at a concentration of 100 mg/mL using an added co-solvent (4% acetone) for that plant extract [the concentration was modified from Ahmad *et al*. (2013)]. 4% acetone (co-solvent) and distilled water were used as negative controls. The mortality data from bioassay was corrected using corrected mortality according to Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\%\ \text{mortality in treatment} - \%\ \text{mortality in control}}{100 - \%\ \text{mortality in control}} \times 100$$

Egg stage

Contact activity

Twenty newly laid house fly eggs (<12 h old) were used per replication. Eggs were transferred into a larval food container (2 cm in height × 5 cm in diameter). 200 µL of plant extract (100 mg/mL) and 200 µL of control were applied to eggs in the separate container. The number of eggs hatched, and abnormal eggs were recorded every 24 and 48 h. The newly hatched larvae were transferred to a new food container (2 cm in height × 5 cm in diameter) to observe their survival rate and the development period. Egg Mortality percentage was calculated using the following formula (Reegan *et al.*, 2015).

$$\text{Egg Mortality percentage} = \frac{\text{Number of unhatched eggs}}{\text{Number of eggs introduced}} \times 100$$

Larval stage

Feeding activity

The methods were modified from Subaharan *et al.* (2021). 5 g of larval food were prepared per replication. 2.5 mL of plant extract (100 mg/mL) and control were sprayed into a larval food container (2 cm in height × 5 cm in diameter). Ten 2nd instar of house fly larvae (2 days old) were examined. The mortality rate of the larvae was recorded every 24 h for 3 days. The survivor larvae were transferred to a new food container to observe their survival rate and the development period.

Pupal stage

Contact activity

The dipping technique was utilized for examining the toxicity (Abdel, 2017) to pupae. Ten house fly pupae (3 days old) per replication were transferred into a cotton muslin bag with a rope (56 cm), then the bag was dipped into 5 mL of plant extract solution (100 mg/mL) and maintained for 40 seconds. The pupae were then transferred to plastic containers (7×9×4 cm). The hatching rate of the pupae was recorded every 24 h for 5 days. Survival, and sex ratio were recorded.

Toxicity Test (LC_{50} and LC_{90})

At each stage of the bioassay experiment, the best potential plant extract was evaluated for LC_{50} and LC_{90} (Subaharan *et al.*, 2021). The house fly immature stage was examined using the same methodology as the bioassay experiment. Plant extract with the greatest potential from each stage was diluted at 0, 25, 50, 75, and 100% concentrations. LC_{50} and LC_{90} from the toxicity bioassay were estimated using probit analysis (Finney, 1971).

GC-MS analysis

The chemical composition of the best potential of plant extracts in each stage of house fly from all experiments was analyzed by gas chromatographic-mass spectrometry analysis at the Center for Scientific and Technological Equipment, Suranaree University of Technology.

GC-MS analysis was performed using the equipment GC Agilent 7890A and MS Agilent 7000.The equipment has column HP-5 capillary column (20 m × 0.18 mm, 0.18 um), Helium at the rate of 1 mL/min was used as carrier gas. 2 µL sample volume was injected. The program was started at 40°C, held for 5 min, ramped at 8°C/min to 200°C, then ramped at 5°C/min to 280°C, and held for 20 min. then save the result as a chromatogram. The identification of components was based on the library of NIST MS Search 2.0.

This work was performed according to the Guidelines for Animal Experimentation of the National Research Council of Thailand and approved by the Animal Ethics Committee of Khon Kaen University, Thailand (IACUC-KKU-15/66).

Data analysis

The Statistix 10 program performed an analysis of variance (ANOVA) on the mean percentage mortality, followed by Fisher's least significant difference (LSD) at $P > 0.05$ (Analytical Software, 2013). A probit analysis of mortality vs. concentration was conducted to estimate lethal concentrations (LC_{50} and LC_{90}).

RESULTS

Ovicidal activity

The results revealed that plant extracts obtained with different solvents resulted in different house fly egg mortality values (Table 2). When extracted using acetone and ethanol solvents, the following plants had strong ovicidal activity: *L. aromatica*, *P. odorata*, and *Ma. esculenta*. In this study, plant extracts from *P. odorata* and *L. aromatica* significantly reduced egg mortality (100%) when acetone was used as a solvent. However, there were no significant differences when comparing the results with the ethanol solvent (Table 2). However, hexane extracts in *M. paniculata* and *C. aurantium* had significantly increased ovicidal activity against houseflies (70.93% and 75.58%, respectively).

The house fly larvae which emerged during all treatments were monitored during their development (Table 3). The results revealed that there was no difference between the control and all plant extracts in the larval and pupal developmental time.

Even though some treatments had lower ovicidal activity, they had significant effects on larval and pupal development. The survival rate from egg to adult was much reduced by four plant extracts from: *Ma. esculenta* (hexane, ethyl acetate, and ethanol extracts), *P. odorata* (hexane, and ethyl acetate extract), *L. aromatica* (ethyl acetate extract), *C. aurantium* (hexane and ethyl acetate extracts), and *Co. esculenta* (ethyl acetate and acetone extracts). These

Table 2. Mortality rate of house fly eggs tested with seven plant extracts

Treatment Plants	Solvent	Accumulated eggs hatching[1/]		% eggs hatching	% egg Mortality	% Corrected mortality
		24 h	48 h			
A. scholaris	Hexane	9.6±2.19^{hi} [2/, 3/]	10.8±1.09^{efg}	54	46	37.20
	Ethyl acetate	10.6±0.54^{gh}	10.6±0.54^{fgh}	53	47	38.37
	Acetone	8.4±2.19^{hijk}	8.4±2.19^{ijk}	42	58	51.16
	Ethanol	13±2.73^{def}	13±2.73^{d}	65	35	24.41
	water	15.4±0.54^{abc}	15.4±0.54^{ab}	77	23	10.46
M. paniculata	Hexane	5±2.73^{mno}	5±2.73^{mn}	25	75	70.93
	Ethyl acetate	7.8±1.09^{ijkl}	7.8±1.09^{ijkl}	39	61	54.65
	Acetone	12±0^{fg}	12±0^{def}	60	40	30.23
	Ethanol	13.6±0.54^{cdef}	13.6±0.54^{bcd}	68	32	20.93
	water	16.4±0.54^{ab}	16.4±0.54^{a}	82	18	4.65
C. aurantium	Hexane	4.2±1.64^{no}	4.2±1.64^{no}	21	79	75.58
	Ethyl acetate	8±0^{ijklm}	8±0^{ijk}	40	60	53.48
	Acetone	13.4±2.19^{cdef}	13.4±2.19^{bcd}	67	33	22.09
	Ethanol	12.6±3.28^{efg}	12.6±3.28^{def}	63	37	26.74
	water	13.4±0.54^{cdef}	13.8±1.09^{bcd}	69	31	19.76
Co. esculenta	Hexane	13±2.73^{def}	13±2.73^{d}	65	35	24.4
	Ethyl acetate	13.8±1.64^{cdef}	13.8±1.64^{bcd}	69	31	19.76
	Acetone	8.4±0.54^{hijk}	8.4±0.54^{ijkl}	42	58	51.16
	Ethanol	13.2±4.38^{cdef}	13.2±4.38^{cd}	66	34	23.25
	water	15.2±3.83^{abcd}	15.2±3.83^{abc}	76	24	11.62
L. aromatica	Hexane	7.2±1.09^{jklm}	7.2±1.09^{jkl}	36	64	58.13
	Ethyl acetate	5.8±1.64^{lmn}	5.8±1.64^{lmn}	29	71	66.27
	Acetone	0±0^{q}	0±0^{q}	0	100	100
	Ethanol	0.8±1.09^{pq}	0.8±1.09^{pq}	4	96	95.34
	water	12.8±1.09^{efg}	12.8±1.09^{de}	64	36	25.58
P. odorata	Hexane	10.6±0.54^{gh}	10.6±0.54^{fgh}	53	47	38.37
	Ethyl acetate	7.4±0.54^{ijkl}	7.4±0.54^{ijkl}	36	63	56.97
	Acetone	0±0^{q}	0±0^{q}	0	100	100
	Ethanol	1.2±1.64^{pq}	1.2±1.64^{pq}	6	94	93.02
	water	7±0^{klm}	8.4±0.54^{ijk}	42	58	51.16
Ma. esculenta	Hexane	9.4±3.28^{hij}	9.4±3.28^{ghi}	47	53	45.34
	Ethyl acetate	6.4±0.54^{klmn}	6.4±0.54^{klm}	32	68	62.79
	Acetone	8.6±0.54^{hijk}	8.6±0.54^{hij}	43	57	50
	Ethanol	2.8±1.09^{op}	2.8±1.09^{op}	14	86	83.72
	water	12.8±1.64^{efg}	12.8±1.64^{de}	64	36	25.58
Control (4% acetone)		14.4±2.19^{bcde}	17±0.70^{a}	85	15	1.16
Control (Distilled water)		17.2±0.44^{a}	17.2±0.44^{a}	86	14	0

[1/]n=20, [2/]Mean±SD, [3/] within each column, Mean±SD followed by similar superscript letters indicate no statistically significant difference (P > 0.05).

treatments also resulted in a lower percentage of female hatching from house fly pupae than male hatching. There is no abnormal adult in the experiment. Lethal concentrations (LC_{50} and LC_{90}) were evaluated for the *L. aromatica* and *P. odorata* (in acetone extract) treatments, which have the most effective ovicidal activity. LC_{50} of *L. aromatica* and *P. odorata* were 42.657, and 7.816 mg/mL, respectively. LC_{90} were 124.738 and 31.117 mg/mL, respectively (Table 7).

Larvicidal activity

The efficiency of seven plant extracts in different solvents was tested on the larval stage of the house fly. The results indicated that most hexane extracts caused a high mortality rate for house fly larvae. The plant extracts of *M. paniculata* (hexane extract) and *C. aurantium* (hexane and ethyl acetate extracts) induced the highest percentage of larval mortality (100%). However, it was not significantly different with *A. scholaris* (hexane and acetone extract), *M. paniculata* (ethyl acetate and acetone extracts), *C. aurantium* (acetone extract), *Co. esculenta* (hexane and ethanol extracts), *P. odorata* (hexane extract), and *Ma. esculenta* (acetone extract). For larvicidal activity, *L. aromatica* (acetone extract) and *P. odorata* (acetone extract) resulted in a low percentage of larval mortality (24.48 and 26.53%) (Table 4).

Survival of house fly larvae after 96 h during all treatments was observed during their development (Table 5). The results showed that the effects of plant extracts extended to the developmental period of the larval and pupal stages. The larval development period was 7–10 days, which was longer than the control period of 5–6 days. For the pupal stage, pupal duration was extended for 1–2 days longer than in the control. Even though some treatments had low larvicidal activity, they had a significant effect on insect development. The larvae treated with plant extracts had significantly lower survival rate than controls when they developed from the larval stage through to the adult stage. Compared to the control group, survival of adults was less than 50%. Adults from all treatments had a higher proportion of females than males. Adult deformity was not present. Lethal concentrations (LC_{50} and LC_{90}) were estimated for the high efficiency treatments in controlling house fly larvae: *M. paniculata* (hexane extract) and *C. aurantium* (hexane and ethyl acetate extract). The results revealed that LC_{50} of those three treatments was 4.865, 11.587, and 13.365, and LC_{90} was 22.284, 37.757, 35.156 mg/mL, respectively (Table 7).

Table 3. Development of house fly after eggs tested with seven plant extracts

Treatment Plants	Solvent	No. larvae (hatched larvae from 20 eggs)[1/]	Mortality rate (3rd)	Larval duration (days)	No. pupae[1/]	Pupal duration (days)	No. adult	Sex ratio M:F	% Total mortality	Corrected mortality
A. scholaris	Hexane	10.8±1.09^{efg}[3/]	2.4±0.54[2/]	5-6	8.4±0.54	5-6	7.4±0.54	1:1.17	63	50
	Ethyl acetate	10.6±0.54^{fgh}	4.6±2.19	5-6	6±2.73	5-6	5.6±3.28	1:1	72	62.12
	Acetone	8.4±2.19^{ijk}	2±0	5-6	6.4±2.19	5-6	6±2.73	1:0.42	70	59.45
	Ethanol	13±2.73^{d}	0±0	5-6	13±2.73	5	11.2±1.64	1:0.33	44	24.32
	water	15.4±0.54^{ab}	0.8±1.09	5-6	14.6±0.54	5-6	12.4±0.54	1:0.51	38	16.21
M. paniculata	Hexane	5±2.73^{mn}	1.4±0.54	5-6	3.6±2.19	5	3.6±2.19	1:0.12	82	75.67
	Ethyl acetate	7.8±1.09^{ijkl}	1.2±1.64	5-6	6.6±0.54	5-6	5.2±1.09	1:1	74	64.86
	Acetone	12±0^{def}	4.8±1.09	5-6	7.2±1.09	5-6	5.6±0.54	1:0.47	72	62.16
	Ethanol	13.6±0.54^{bcd}	8±0	5-6	5.6±0.54	5-6	5.2±1.09	1:0.36	74	64.86
	water	16.4±0.54^{a}	8±0	5-6	8.4±0.54	5-6	5.4±0.54	1:0.58	73	63.51
C. aurantium	Hexane	4.2±1.64^{no}	2.6±0.54	5-6	1.6±2.19	5	1.6±2.19	1:1	92	89.18
	Ethyl acetate	8±0^{ijk}	3.4±0.54	5-6	4.6±0.54	5-6	2.8±1.09	1:0.4	86	81.08
	Acetone	13.4±2.19^{bcd}	4.2±1.64	5-6	9.2±3.83	5-6	6.2±1.09	1:1.21	69	58.10
	Ethanol	12.6±3.28^{def}	2±0	5-6	10.6±3.28	5-6	9.2±3.83	1:0.39	54	37.83
	water	13.8±1.09^{bcd}	2±0	5-6	11.8±1.09	5-6	9±0	1:0.40	55	39.18
Co. esculenta	Hexane	13±2.73^{d}	3.6±0.54	5-6	9.4±2.19	5-6	7.8±1.64	1:0.85	61	47.29
	Ethyl acetate	13.8±1.64^{bcd}	10.4±0.54	5-6	3.4±2.19	5-6	2.8±1.64	1:0.75	86	85.13
	Acetone	8.4±0.54^{ijk}	6.6±0.54	5-6	1.8±0.44	5-6	1.8±0.44	1:1	91	87.83
	Ethanol	13.2±4.38^{cd}	6.6±2.19	5-6	6.6±2.19	5-6	5.4±0.54	1:0.42	73	63.51
	water	15.2±3.83^{abc}	5.8±4.38	5-6	9.4±0.54	5-6	7.2±1.64	1:1.11	64	51.35
L. aromatica	Hexane	7.2±1.09^{jkl}	2.6±2.19	5-6	4.6±3.28	5-6	4±2.73	1:0.33	80	72.97
	Ethyl acetate	5.8±1.64^{lmn}	4.6±0.54	5-6	1.2±1.09	5-6	1.2±1.09	1.2:0	94	91.89
	Acetone	0±0^{q}	–	–	–	–	–	–	100	100
	Ethanol	0.8±1.09^{pq}	0.8±1.09	–	–	–	–	–	100	100
	water	12.8±1.09^{de}	3±0	5-6	9.8±1.09	5-6	8.6±2.19	1:1.26	57	41.89
P. odorata	Hexane	10.6±0.54^{fgh}	7.6±0.54	5-6	3±0	5-6	3±0	1:0.66	85	79.72
	Ethyl acetate	7.4±0.54^{ijkl}	4.8±1.09	5-6	2.6±0.54	5-6	2.6±0.54	1:3.33	87	82.43
	Acetone	0±0^{q}	–	–	–	–	–	–	100	100
	Ethanol	1.2±1.64^{pq}	1.2±1.64	–	–	–	–	–	100	100
	water	8.4±0.54^{ijk}	3.6±0.54	5-6	4.8±1.09	5-6	4±0	1:0.66	80	72.97
Ma. esculenta	Hexane	9.4±3.28^{ghi}	6.2±3.49	5-6	3.2±0.44	5-6	2.6±3.28	1:0.25	85	79.72
	Ethyl acetate	6.4±0.54^{klm}	5.2±1.64	5-6	1.2±1.09	5-6	1.2±1.09	1:1	94	91.89
	Acetone	8.6±0.54^{hij}	4.4±0.54	5-6	4.2±1.09	5-6	3.6±0.54	1:0.38	82	75.67
	Ethanol	2.8±1.09^{op}	0.6±0.54	5-6	2.2±1.64	5	2.2±1.64	2.2:0	89	85.13
	water	12.8±1.64^{def}	3.2±1.09	5-6	9.6±0.54	5-6	7.2±1.09	1:0.56	64	51.35
Control (4% acetone)		17±0.70^{a}	0.8±1.09	5-6	16.2±1.30	5-6	14.4±0.54	1:1.16	28	2.70
Control (Distilled water)		17.2±0.44^{a}	0.6±3.48	5-6	16.6±1.14	5-6	14.8±1.09	1:1.05	26	0

[1/]n=20, [2/]Mean±SD, [3/] within each column, Mean±SD followed by similar superscript letters indicate no statistically significant difference (P > 0.05).

Pupicidal activity

Pupal mortality was found to be impacted by plant extracts in various solvents. *C. aurantium* (ethyl acetate extract), *Ma. esculenta* (hexane extract), and *Co. esculenta* (hexane extract) completely killed house fly pupa, followed, in decreasing rank of efficiency, by *M. paniculata* (ethanol extract), *C. aurantium* (acetone extract), *P. odorata* (hexane and ethyl acetate extract), and *Ma. esculenta* (acetone extract) (Table 6). LC_{50} of *C. aurantium* (ethyl acetate extract), *Ma. esculenta* (hexane extract), and *Co. esculenta* (hexane extract) was 26.424, 30.408, 37.411, and 61.801, respectively, and LC_{90} was 61.801, 72.945, and 100, respectively (Table 7). Plant extracts did not affect pupal duration. Adult males were in a higher proportion than females after applying these plant extracts during the pupal stage. (Table 6).

GC-MS Analyses

The best potential plant extracts at each stage of the bioassay experiment were analyzed by GC-MS. The major chemical components (>4%) from each plant extract were shown in Table 8. According to the results of acetone extracts of *L. aromatica*, flavonoids were the majority of the compound (50.72%), followed by acyclic diterpenoids and fatty acids respectively. Five major components were identified in acetone leaf extracts of *P. odorata*. Four components were identified as stigmastane, olefinic compounds, acyclic diterpenoids, and fatty acids. One chemical remained unidentified. The main component in *C. aurantium* acetone and ethyl acetate extracts was found to be alkane (13.22% and 12.3%, respectively). The majority of the chemical compounds in these extracts belonged to the terpene chemical classes (4 chemical compounds for each extraction). Hexane extracts of *M. paniculata* included eight major components, four of which were categorized as terpenes, one of which was classified as an alkene, vitamin, and pesticide, and one of which was unidentified. From *Co. esculenta* hexane extract, 16-hentriacontanone (ketone group) had the largest percentage at 46.47%. It was followed by β-sitosterol (stigmastane) at 15.23% and phytol (acyclic diterpenoids) at 5.10%. There are nine main components in the hexane extract of *Ma. esculenta*. Seven of them were terpene groups, one was vitamin E, and one of them was stigmastane.

Table 4. Mortality rate of house fly larvae tested with seven plant extracts by feeding method

Treatment Plants	Solvent	Cumulative mortality rate[1/]			% Total mortality	Corrected mortality
		24 h	48 h	72 h		
A. scholaris	Hexane	6±1.41^{efgh} **2/**, 3/	8.6±2.19^{abcde}	8.6±2.19^{abc}	86	85.61
	Ethyl acetate	6±2.23^{efgh}	7.6±0.54^{def}	7.6±0.54^{cde}	76	75.51
	Acetone	3.4±0.89^{ijk}	6.8±1.64^{efg}	8.4±2.30^{abcd}	84	83.67
	Ethanol	7.4±1.34^{cdef}	7.4±1.34^{def}	7.4±1.34^{cde}	74	73.46
	water	2.8±1.64^{jklm}	2.8±1.64^{jk}	3.6±0.89^{ijkl}	36	34.69
M. paniculata	Hexane	8.6±0.89^{abc}	9.8±0.44^{ab}	10±0^{a}	100	100
	Ethyl acetate	5.6±2.19^{gh}	9.4±0.89^{abc}	9.6±0.54^{ab}	96	95.91
	Acetone	8.2±1.30^{abcd}	8.4±2.30^{bcd}	9.4±1.34^{ab}	94	93.87
	Ethanol	6.6±0.54^{efgh}	6.8±0.44^{efg}	6.8±0.44^{def}	68	67.34
	water	2±0^{klmn}	2.6±0.54^{jk}	5.6±0.89^{fg}	56	55.10
C. aurantium	Hexane	9.6±0.54^{a}	10±0^{a}	10±0^{a}	100	100
	Ethyl acetate	8.4±0.89^{abc}	10±0^{a}	10±0^{a}	100	100
	Acetone	8.6±0.54^{abc}	9.6±0.54^{abc}	9.6±0.54^{ab}	96	95.91
	Ethanol	3.4±0.89^{ijk}	3.6±0.89^{ijk}	5.4±0.89^{fgh}	54	53.06
	water	2.6±0.54^{jklm}	2.6±0.54^{jk}	4.6±1.94^{ghijk}	46	44.89
Co. esculenta	Hexane	6.2±1.30^{efgh}	9.4±0.89^{abc}	9.4±0.89^{ab}	94	93.87
	Ethyl acetate	7.4±0.89^{bcde}	7.6±0.54^{def}	7.6±0.54^{bcd}	76	75.51
	Acetone	5.8±1.78^{fgh}	6.2±1.30^{fgh}	6.2±1.30^{efg}	62	61.22
	Ethanol	9.2±1.78^{ab}	9.2±1.78^{abc}	9.4±1.34^{ab}	94	93.87
	water	1.2±1.09^{mno}	2.2±1.09^{klm}	7±2.82^{cdef}	70	69.38
L. aromatica	Hexane	8.4±2.19^{abc}	8.4±2.19^{bcd}	8.4±2.19^{abcd}	84	83.67
	Ethyl acetate	7.4±0.89^{cdef}	7.4±0.89^{def}	8±1.41^{bcd}	80	79.59
	Acetone	1.4±0.89^{lmno}	2.6±0.54^{jk}	2.6±0.54^{l}	26	24.48
	Ethanol	2.2±1.30^{jklmn}	2.4±1.34^{kl}	3.2±1.09^{jkl}	32	30.61
	water	3±1.41^{ijkl}	3.4±0.89^{jk}	3.8±0.44^{hijkl}	38	36.73
P. odorata	Hexane	8.4±0.89^{abc}	8.4±0.89^{bcd}	8.4±0.89^{abcd}	84	83.67
	Ethyl acetate	4.6±0.89^{hi}	5.6±0.54^{gh}	7.4±1.34^{cde}	74	73.46
	Acetone	1.4±0.89^{lmno}	2.2±1.30^{klm}	2.8±1.78^{l}	28	26.53
	Ethanol	3.2±2.48^{ijk}	4±1.41^{ij}	4.8±1.64^{ghij}	48	46.93
	water	0.6±0.54^{no}	0.8±0.44^{mn}	3±1.73^{kl}	30	28.57
Ma. esculenta	Hexane	4.6±0.54^{hi}	5.8±0.44^{gh}	8±0^{bcd}	80	79.59
	Ethyl acetate	7.4±1.34^{cdef}	8.2±1.78^{cde}	8.2±1.78^{bcd}	82	80
	Acetone	7±1.41^{cdefg}	8.4±0.89^{bcd}	9.6±0.54^{ab}	96	95.91
	Ethanol	3.8±2.16^{ij}	5±1.73^{hi}	6.2±2.68^{efg}	62	61.22
	water	0.8±0.44^{no}	1±0^{lmn}	5±1.41^{ghi}	50	48.97
Control (4% acetone)		0±0^{o}	0.2±0.44^{n}	0.2±0.44^{m}	2	0
Control (Distilled water)		0±0^{o}	0.2±0.44^{n}	0.2±0.44^{m}	2	0

[1/]n=10, [2/]Mean±SD, [3/] within each column, Mean±SD followed by similar superscript letters indicate no statistically significant difference (P > 0.05).

DISCUSSION

The result of the experiment showed that bioactive compounds from each plant extract were dependent on solvent in the extraction process. The results of the experiment showed that the various phases of the house fly were impacted differently by each plant that was extracted using a different solvent. Abdullahi and Haque (2020) and Pinelo *et al*. (2004) reported that the solvent was the main factor in the extraction of biochemical compounds from plants. Various compounds were extracted in solvents of different polarity (Pandey & Tripathi, 2014). For instance, Alkaloids interact well in non-polar solvents, whereas saponins, polyphenols and tannins interact well in polar solvents (Azmir *et al*., 2013; Dai & Mumper, 2010). It is impossible to extract all of the bioactive chemicals from plant material using a single solvent.

The acetone and ethanol solvent-based extracts (polar solvents) of *P. odorata* and *L. aromatica* resulted in a significant mortality rate of egg stage (Table 2). However, there were no good results from the other solvent extractions of either plant. Do *et al*. (2014) found that ethanol and acetone extracts of *L. aromatica* contain high phenolic content, which is consistent with the high flavonoid (50.72%) values in the phenolic group in this investigation (Table 8). Nguyen *et al*. (2020) reported that ethanolic extract from the leaves of *P. odorata* showed high total flavonoid (70.65 ± 4.14 µg/mg) and phenolic content (58.56 ± 3.86 µg/mg). Previous research indicates that flavonoid components may be significant bioactive substances for ovicidal activity in polar solvent-based extracts of *P. odorata* and *L. aromatica* (Valizadeh *et al*., 2021). According to Valizadeh *et al*. (2021), the phenolic compounds in essential oils have an impact on the embryo's movement and vital systems in the beetle [*Xanthogaleruca luteola* (Mull.)]. They also inhibit gas exchange inside the egg, which causes the crust to harden and directly affects protoplasm, which results in the embryo dying inside the egg. Flavonoids are suggested to disturb the embryogenesis process (Rajkumar & Jebanesan, 2009). In mosquitoes, flavonoid compounds are effective ovicides in the early stages of egg development because the eggshell is very thin at this stage, which facilitates entry into the egg (Rajkumar & Jebanesan, 2009). In addition to flavonoids, the *L. aromatica* acetone extract contained a total of 11% terpenoids concentration.

Table 5. Development of house fly after larvae tested with seven plant extracts by feeding method

Treatment Plants	Solvent	Survival rate	Larval duration (days)	Number of pupae[1/]	Pupal duration (days)	No. adult	Sex ratio M:F	% Total mortality	Corrected mortality
A. scholaris	Hexane	1.4±2.19$^{klm3/}$	7-8	0.8±1.09[2/]	5-6	0.8±1.09	1:1	92	91.66
	Ethyl acetate	2.4±0.54^{ijk}	9-10	2.4±0.54	5-6	1.8±0.44	9:0	82	81.25
	Acetone	1.6±2.30^{jklm}	8-9	0.8±1.09	5-6	0.6±0.89	1:0.5	94	93.75
	Ethanol	2.6±1.34^{ijk}	9-10	1.8±1.30	5-6	1.2±1.78	1:0.5	88	87.5
	water	6.4±0.89^{bcde}	9-10	4.8±1.78	6-7	4.4±1.34	1:0.57	56	54.16
M. paniculata	Hexane	–	–	–	–	–	–	100	100
	Ethyl acetate	0.4±0.54^{lm}	8-9	0.4±0.54	5-6	0.4±0.54	2:0	96	95.83
	Acetone	0.6±1.34^{lm}	9-10	0.4±0.89	5-6	0.4±0.89	1:1	96	95.83
	Ethanol	3.2±0.44^{hij}	7-8	3.2±0.44	6-7	2.2±0.44	1:0.57	78	77.08
	water	4.4±0.89^{gh}	7-8	4±0.70	6-7	3.4±0.89	1:0.54	66	64.58
C. aurantium	Hexane	–	–	–	–	–	–	100	100
	Ethyl acetate	–	–	–	–	–	–	100	100
	Acetone	0.4±0.54^{im}	8-9	–	–	–	–	100	100
	Ethanol	4.6±0.89^{fgh}	8-9	3.8±0.44	6-7	2.8±0.44	1:0.27	72	70.83
	water	5.4±1.94^{cdefg}	7	5±1.41	6-7	4.4±0.89	1:0.57	56	54.16
Co. esculenta	Hexane	0.6±0.89^{lm}	8-9	0.4±0.54	5-6	0.2±0.44	1:0	98	97.916
	Ethyl acetate	2.4±0.54^{jkl}	8-9	1.4±0.54	5-6	0.6±0.89	1:0.5	96	95.83
	Acetone	3.8±1.30^{ghi}	8-9	2.4±2.19	5-6	2.2±2.16	1:0.37	78	77.08
	Ethanol	0.6±1.34^{lm}	8-9	0.6±1.34	7-8	0.4±0.89	1:1	96	95.83
	water	3.0±2.82^{hijk}	7	3±2.82	6-7	2.2±1.78	1:1.2	78	77.08
L. aromatica	Hexane	1.6±2.19^{jklm}	7-8	1.6±2.19	5-6	1.2±1.78	6:0	88	87.5
	Ethyl acetate	2±1.41^{jkl}	7-8	2±1.41	5-6	1.8±1.09	1:0.8	82	81.25
	Acetone	7.4±0.54^{b}	9	7.4±0.54	5-6	4.2±0.44	1:0.4	58	56.25
	Ethanol	6.8±1.09^{bcd}	8-9	5.2±2.48	7-8	4.4±3.57	1:1.2	56	54.16
	water	6.2±0.44^{bcdef}	7	6.2±0.44	6-7	5±1.87	1:0.66	50	47.91
P. odorata	Hexane	1.6±0.89^{jklm}	9-10	1.2±0.44	5-6	0.2±0.44	1:0	98	97.91
	Ethyl acetate	2.6±1.34^{ijk}	9-10	2.4±0.89	5-6	0.8±0.44	1:3	92	91.66
	Acetone	7.2±1.78^{b}	9-10	6.2±0.44	5-6	1.6±2.60	1:0.14	84	83.33
	Ethanol	5.2±1.64^{defg}	7-8	4.2±1.09	6-7	3.2±1.78	1:0.6	68	66.66
	water	7.0±1.73^{bc}	9	7±1.73	6-7	6.2±0.44	1:0.55	38	35.41
Ma. esculenta	Hexane	2.0±0^{jkl}	9-10	1.8±0.44	5-6	0.8±0.44	4:0	92	91.66
	Ethyl acetate	1.8±1.78^{jkl}	9-10	1.8±1.78	5-6	0.4±0.54	1:1	96	95.83
	Acetone	0.4±0.54^{lm}	7-8	0.4±0.54	5-6	0.2±0.44	1:0	98	97.91
	Ethanol	3.8±2.68^{ghi}	9	3.2±3.19	7-8	1.8±2.38	1:1.25	82	81.25
	water	5.0±1.41^{efg}	7-8	5±1.41	6-7	3.6±1.14	1:0.8	64	62.5
Control (4% acetone)		9.8±0.44^{a}	5-6	9.8±0.44	5-6	9.6±0.54	1:1.66	4	0
Control (Distilled water)		9.8±0.44^{a}	5-6	9.8±0.44	5-6	9.6±0.54	1:1.76	4	0

[1/]n=10, [2/]Mean±SD, [3/] within each column, Mean±SD followed by similar superscript letters indicate no statistically significant difference (P > 0.05).

Terpenoids content was as well detected from the acetone extract of *P. odorata* in this experiment (7.22%). The results related to the report of Sasongko *et al.* (2011) that *P. odorata* has a significant amount of caryophyllene (a member of the terpene group), which may be extracted using an acetone solvent. Dambolena *et al.* (2016) reported that terpenes from some essential oils have an ovicidal effect on lice. Chemical components from ethanol extract in *L. aromatica* were not evaluated in this study. Soeung *et al.* (2022) revealed that high saponin content was determined in the ethanol extract in *L. aromatica*. The mode of action of saponin in the insect egg has not been described, although it has been reported to affect the ovicidal activity of many insects, such as the corn borer [*Ostrinia furnacalis* (Guenee)] (Liu *et al*., 2019) and *Culex pipiens* (Djeghader *et al*., 2018). Even though other treatments have less of an effect on house fly eggs, they have an impact on the growth and development of the larvae that hatch from a surviving egg. Incomplete development during the larval stage might be due to abnormalities in embryogenesis.

Based on the results from larvicidal bioassay, house fly larvae died at a significant rate when exposed to most plant extracts that use hexane as a solvent. The plant extracts with the highest percentage of larval death (100%) were *M. paniculata* (hexane extract) and *C. aurantium* (hexane and ethyl acetate extract) (Table 4). Analysis of bioactive compounds in plant extracts revealed that the major components extracted from these 2 plants were terpenoids and alkanes (Table 8). In previous studies, alkanes were reported to have insecticidal activity against insect larvae, including *Plutella xytostella* L. larvae in their second instar (Poonsri *et al*., 2015) and *Spodoptera litura* (F.), *Spodoptera exigua* (H bner), and *Plutella xylostella* L. in their third instar (Junhirun *et al*., 2018). According to Junhirun *et al.* (2018), alkanes have been reported to be both a contact toxin and a feeding deterrent. Sonter *et al*. (2021) reported that the main ingredient in *M. paniculata* derived from hexane extraction is cyclohexane. It's a monocyclic monoterpene, or terpenoid (Zielińska-Błajet *et al*., 2021). This related to the results of this study, terpene was the major component of

Table 6. Mortality rate of house fly pupae after tested with seven plant extracts by dipping method

Treatment Plants	Solvent	Accumulated pupal hatching[1/]					No. adult	% Total mortality	Corrected mortality	Age of pupa (days)	Sex ratio M:F
		24 h	48 h	72 h	96 h	120 h					
A. scholaris	Hexane	0±0	0±0[2/]	1.6±0.54$^{de3/}$	6.2±1.09^{a}	7.6±0.54^{ab}	76	24	11.627	6-8	1:0.72
	Ethyl acetate	0±0	0±0	1.6±0.54^{de}	5.2±1.09^{ab}	5.2±1.09^{ef}	52	48	39.534	6-7	1:0.44
	Acetone	0±0	0±0	3.6±0.54^{b}	5.6±0.54^{ab}	6.6±0.54^{bcd}	66	34	23.255	6-8	1:0.94
	Ethanol	0±0	0±0	1±0^{ef}	4±0^{cdef}	4±0^{g}	40	60	53.488	6-7	1:0.05
	water	0±0	0±0	0.4±0.54^{fg}	2±0h^{hij}	7.6±0.54^{ab}	76	24	11.627	6-8	1:0.58
M. paniculata	Hexane	0±0	0±0	2.2±0.44^{cd}	3±0^{fgh}	4.6±0.54^{fg}	46	54	46.511	6-8	1:1.87
	Ethyl acetate	0±0	0±0	0±0^{g}	1.6±0.54^{ijk}	1.6±0.54^{jklm}	16	84	81.395	7	1:0.6
	Acetone	0±0	0±0	0±0^{g}	1.8±1.64^{ij}	2.4±2.19^{ij}	24	76	72.093	7-8	1:1
	Ethanol	0±0	0±0	0±0^{g}	0±00^{l}	0.6±0.54^{mn}	6	94	93.023	8	1:2
	water	0±0	0±0	2.6±0.54^{c}	4±0^{cdef}	7.6±0.54^{ab}	76	24	11.627	6-8	1:0.35
C. aurantium	Hexane	0±0	0±0	0.4±0.54^{fg}	3.6±0.54^{efg}	5.6±0.54^{def}	56	44	34.883	6-8	1:1.15
	Ethyl acetate	0±0	0±0	0±0^{g}	0±0^{l}	0±0^{n}	0	100	100	-	-
	Acetone	0±0	0±0	0±0^{g}	0.6±0.54^{kl}	0.6±0.54^{mn}	6	94	93.023	7-8	1:2
	Ethanol	0±0	0±0	0±0^{g}	0.6±0.54^{kl}	1.2±1.09^{klm}	12	88	86.046	7-8	1:1
	water	0±0	0±0	0.4±0.54^{fg}	3.6±0.54^{efg}	3.6±0.54^{gh}	36	64	58.139	6-8	1:1.25
Co. esculenta	Hexane	0±0	0±0	0±0^{g}	0±0^{l}	0±0^{n}	0	100	100	-	-
	Ethyl acetate	0±0	0±0	1±0^{ef}	2.2±1.09^{hi}	2.2±1.09^{ijk}	22	78	74.418	7	1:0.83
	Acetone	0±0	0±0	0±0^{g}	0.6±0.54^{kl}	1±0^{lmn}	10	90	88.372	7-8	1:0.66
	Ethanol	0±0	0±0	0±0^{g}	0±0^{l}	1±0^{lmn}	10	90	88.372	8	1:0.66
	water	0±0	0±0	1.8±1.64^{cde}	3±0^{fgh}	3.6±0.54^{gh}	36	64	58.139	6-8	1:1.25
L. aromatica	Hexane	0±0	0±0	0±0^{g}	0±0^{l}	2.6±0.54^{hij}	26	74	69.767	8	1:0.62
	Ethyl acetate	0±0	0±0	0±0^{g}	1.6±0.54^{ijk}	2±0^{ijkl}	20	80	76.744	7-8	1:0.66
	Acetone	0±0	0±0	0±0^{g}	1.8±1.64^{ij}	2.8±1.64^{hi}	28	72	67.441	6-8	1:1.8
	Ethanol	0±0	0±0	3.8±1.64^{b}	4.6±0.54^{bcde}	6.2±1.09^{cde}	62	38	27.906	6-8	1:0.47
	water	0±0	0±0	1±0^{ef}	1±0^{jkl}	1±0^{lmn}	10	90	88.372	6	1:0.25
P. odorata	Hexane	0±0	0±0	0±0^{g}	0.4±0.54^{l}	0.6±0.54^{mn}	6	94	93.023	7-8	0:3
	Ethyl acetate	0±0	0±0	0±0^{g}	0.6±0.54^{kl}	0.6±0.54^{mn}	6	94	93.023	7-8	1:2
	Acetone	0±0	0±0	5±2.73^{a}	5±2.73^{bc}	6.8±2.68^{bc}	68	32	20.930	7-8	1:0.61
	Ethanol	0±0	0±0	1.6±0.54^{de}	4.8±1.64^{bcd}	5.6±0.54^{def}	56	44	34.883	6-8	1:4.6
	water	0±0	0±0	2.6±0.54^{c}	3.8±1.64^{def}	6.6±0.64^{bcd}	66	34	23.255	6-8	1:0.83
Ma. esculenta	Hexane	0±0	0±0	0±0^{g}	0±0^{l}	0±0^{n}	0	100	100	-	-
	Ethyl acetate	0±0	0±0	0±0^{g}	1.8±1.64^{ij}	2.4±1.34^{ij}	24	76	72.093	7-8	1:0.71
	Acetone	0±0	0±0	0.6±0.54^{fg}	0.6±0.54^{kl}	0.6±0.54^{mn}	6	94	93.023	6-8	1:2
	Ethanol	0±0	0±0	0±0^{g}	0.6±0.54^{kl}	1.2±1.09^{klm}	12	88	86.046	7-8	1:1
	water	0±0	0±0	2.6±0.54^{c}	2.6±0.54^{ghi}	2.8±0.44^{hi}	28	72	67.441	6-8	1:1.8
Control (4% acetone)		0±0	0±0	1.6±0.54^{de}	4.6±0.54^{bcde}	8.4±0.54^{a}	84	16	2.325	6-8	1:1.80
Control (Distilled water)		0±0	0±0	2±0^{cd}	1.6±0.54^{ijk}	8.6±0.54^{a}	86	14	0	6-8	1:1.86

[1/]n=10, [2/]Mean±SD, [3/] within each column, Mean±SD followed by similar superscript letters indicate no statistically significant difference (P > 0.05).

Table 7. Lethal concentration ($LC_{50,}$ LC_{90}) of plant extract against immature stage of house flies

Stage	Plants	Solvent	LC_{50}[1/] (mg/mL)	LC_{90} (mg/mL)
Eggs	*L. aromatica*	Acetone	42.657[2/]	124.738
	P. odorata	Acetone	7.816	31.117
Larvae	*C. aurantium*	Hexane	11.587	37.757
	C. aurantium	Ethly acetate	13.365	35.156
	M. paniculata	Hexane	4.865	22.284
Pupa	*C. aurantium*	Ethly acetate	26.424	61.801
	Co. esculenta	Hexane	37.411	100.000
	Ma. esculenta	Hexane	30.408	72.945

[1/]Lethal concentration is indicated with 95% confidence limit (CL,), [2/]LC_{50} and LC_{90} of plant extract mg/mL.

C. aurantium extracted by hexane. According to the findings, terpene is also the main component of *C. aurantium* that is extracted using ethyl acetate. D-limonene (a monoterpene substance) is the most active constituent (94%) from leaves of *C. aurantium* extracted by hexane (Maksoud *et al*., 2021; Changbunjong *et al.*, 2022). However, in this study Alkane, Eucalyptol, α-Amyrin and α-Terpineol were the main components. Many factors, including plant organs, phenological stage, genetic profile, and environmental abiotic and biotic factors including growing site, light, temperature, radiation, soil salinity and dryness, infections, and herbivore attacks, can cause a large variation in the concentration of bioactive chemicals (Cirak & Radusiene 2019). Kuppusamy and Murugan (2010) reported that α-Amyrin action similar to juvenile hormone analogs in combination with growth regulator activity and toxicity in the larvae of *Anopheles Stephensi* Liston. Huang *et al*. (2022) reported that terpineol was found to have insecticidal effect against *P. xylostella* and has been correlated to decreased activity of GST, CAT, AChE, and Na+/

Table 8. GC-MS analysis of bioactive compounds from plant extracts

Plants (solvent)	Main compound	Peak area %	Class of compound	Chemical group
L. aromatica	Flavone, 5,7-dihydroxy-3',4',5'-trimethoxy-	50.72	Flavonoids	Polyphenolic compound
(Acetone)	Phytol	6.21	Acyclic diterpenoids	Terpenoids
	n-Hexadecanoic acid	5.29	Fatty acid	Fatty acid
	Phytol, acetate	5.10	Acyclic diterpenoids	Terpenoids
P. odorata	β-Sitosterol	11.88	Stigmastane and derivatives	Organic compounds
(Acetone)	2,5-Furandione, 3-dodecyl-	10.31	–	–
	3-Penten-2-one, 4-methyl-	8.63	Olefinic compound	–
	Phytol, acetate	7.22	Acyclic diterpenoids	Terpenoids
	n-Hexadecanoic acid	8.92	Fatty acid	Fatty acid
C. aurantium	Hentriacontane	15.62	Alkane	Alkane
(Hexane)	Eucalyptol	10.98	Monoterpenes	Terpene
	dl-α-Tocopherol	9.12	vitamin E	vitamin
	α-Amyrin	8.79	Pentacyclic triterpenoid	Terpenoids
	α-Terpineol	4.87	Menthane monoterpenoids	Terpenoids
	β-Linalool	4.01	Acyclic monoterpenoid	Terpenoids
C. aurantium	Hentriacontane	13.22	Alkane	Alkane
(Ethyl acetate)	α-Amyrin	12.64	Triterpenoids	Terpenoids
	dl-α-Tocopherol	9.79	vitamin E	vitamin
	Eucalyptol	7.53	Monoterpenes	Terpene
	β-Sitosterol	4.58	Stigmastane and derivatives	Organic compounds
	α-Terpineol	4.46	Menthane monoterpenoids	Monoterpenoids
	Phytol	4.42	Acyclic diterpenoids	Terpenoids
	n-Hexadecanoic acid	4.04	Fatty acid	Fatty acid
M. paniculata	7-Geranyloxycoumarin	12.30	Coumarins	Terpene
(Hexane)	β-Caryophyllene	8.71	Sesquiterpene	Terpenes
	Cycloeucalenol acetate	8.36	Puerarin	Isoflavone glycoside group
	6-tert-Butyl-1-Tetralone	6.56	–	–
	Binapacryl	5.59	Pesticides	Pesticides
	Hentriacontane	5.22	Alkane	Alkane
	Germacrene D	4.69	Sesquiterpenoids	Terpenoids
	dl-α-Tocopherol	4.37	vitamin E	Vitamin
Co. esculenta	16-Hentriacontanone	46.67	Dialkyl ketone	Ketone
(Hexane)	β-Sitosterol	15.23	Stigmastane and derivatives	Organic compounds
	Phytol	5.10	Acyclic diterpenoids	Terpenoids
Ma. esculenta	Squalene	18.17	Triterpenoids	Terpenoids
(Hexane)	Friedelan-3-one	13.13	Pentacyclic triterpenoid	Terpenoids
	Lupenone	11.52	Pentacyclic triterpenoid	Terpenoids
	dl-α-Tocopherol	9.00	vitamin E	Vitamin
	Lupeol	8.04	Pentacyclic triterpenoid	Terpenoids
	D-Friedoolean-14-en-3-one	7.05	Pentacyclic triterpenoid	Terpenoids
	β-Amyrin	6.23	Pentacyclic Triterpenoids	Terpenoids
	β-Sitosterol	5.62	Stigmastane and derivatives	Organic compounds
	α-Amyrin	4.93	Pentacyclic Triterpenoids	Terpenoids

K+-ATPase. The effects of eucalyptol were evaluated against the house fly, *M. domestica*, and blow fly, *Chrysomya megacephala* (F.) According to Sukontason *et al*. (2004), eucalyptol had a low larvicide impact (LD50 = 642 µg/µL) against *C. megacephala* but a moderate larvicide effect (LD50 = 101 µg/µL) on *M. domestica* larvae. According to studies by Picollo *et al*. (2008), The cause of eucalyptol's toxicity to various insects is the inhibition of acetylcholinesterase (AChE) enzyme activity, which affects the insect's nervous system and results in paralysis and death.

The results of this investigation indicated that the plant extract influences house fly larval survival and lengthens their developmental period, but not the duration of the pupal stage, which was consistent with the findings of Nisar *et al*. (2021). Due to their ability to delay larval development, some bioactive components found in plant extracts negatively impact insect larvae, such as *S. frugiperda* (Silva *et al*., 2016). In A*nopheles gambiae,* pre-emergence impacts include prolonging the life of its larvae and pupae, preventing molting in these stages, and maybe even causing mortality during the melanization and molting processes. The molting process can cause hormonal imbalances, which can lead to developmental disruptions (Muema *et al.*, 2016).

Akbar *et al*. (2022) suggested that plant extract components enter insects through their integument. The hydrophilic-hydrophobic structure of the cuticle has been reported to involve the penetration of pesticides. Chemical properties of active principals and polarity influence passage of bioactive compounds, like insecticides through the cuticle. Nonpolar molecular mobility is enhanced by the outermost lipophilic layer of the cuticle. Shaaban and Al-Malah (1993) suggested that plant extract may come into contact with the body's surface, allowing chemical compounds to enter the insect's body (the elastic area) and leading the insect to become paralyzed and die.

For house fly pupa, the results revealed that some plant extracts caused high mortality (>80%) (Table 6); such as extracts of *M. paniculata* (ethyl acetate, ethanol extract), *C. aurantium* (ethyl acetate, ethanol, and acetone extract), *Co. esculenta* (acetone,

hexane and ethanol extracts), *L. aromatica* (water), *P. odorata* (ethyl acetate and hexane extracts), and *Ma. esculenta* (hexane ethanol and acetone). Ethyl acetate extract of *C. aurantium* and hexane extract of *Co. esculenta* and *Ma. esculenta* showed 100% mortality of house fly pupae. An alkane function group, hentriacontane compound, was detected with the highest peak area of 15.62% obtained using gas chromatography mass spectrometry in *C. aurantium* extract by ethyl acetate extract. Whereas, terpenoid contents were detected in *Ma. esculenta* extract by hexane. According to Dambolena *et al.* (2016), terpene functioned in the pupal stage by extending the durations that pupae developed as well as decreasing the longevity and fertility of the emerging female adults. 16-Hentriacontanone was the major compound in *Co. esculenta* extract by hexane. Pratiwi *et al*. (2020) revealed that 16-Hentriacontanone was neurotoxic against insects. it was an acetylcholinesterase receptor inhibitor.

In the house fly, the exarate pupa is enclosed in a puparium, which is called a coarctate pupa. The outer cuticular sheet is heavily chitinized puparium, which makes it difficult for chemical compounds to enter. However, on the puparium surface, there are small openings for communication between the pupal tracheal system and the outside air, such as respiratory horns on the fifth segment of the puparium, anterior spiracle on the lateral surfaces of the prothorax at the anterior tip of the puparium and a pair of posterior spiracles on the caudal segment. (Karandika & Ranade, 1964; Siriwattanarungsee *et al*., 2005). Plant extract may penetrate the pupa *via* these tiny pores. In this study, plant extract did not affect duration time of pupa. Therefore, plant extract may have a low effect on house fly pupae in their development. Adult females were in lower proportion than males after application some of the plant extracts during the larval and pupal stage. Rodr□guez-Munoz *et al*. (2019) suggested that in insect populations with a male-biased sex ratio, individuals may spend less in reproduction as a result of focusing more of their energy on mating competition. Population growth is believed to be encouraged by the female-biased sex ratio. Compton and Tu (2022) reported that the desired sex ratio may change due to selection, but sex ratio distortion (SRD) may be caused by meiotic drive and endosymbiont manipulation. Zhang *et al.* (2016) revealed that gossypol, a secondary metabolite from cotton, suppressed *Buchnera aphidicola* (obligate endosymbiont) populations in *Aphis gossypii* Glover populations from both cotton and cucumber. According to this research, plant extract may affect sex determination by reducing the endosymbiont population, which is associated with sex ratio. However, the evidence for these investigated plants has not been published, though.

CONCLUSIONS

Each plant extract's bioactive components varied across different solvents used during the extraction process. The experiment discovered that each plant extract affected the house fly stages differently, depending on the solvent used. Strong ovicidal activity was observed in plant extracts in high polarity solvents (ethanol and acetone). Conversely, low polarity solvent (hexane) extracts exhibited strong larvicidal and pupicidal activity during the larval and pupal phases. A significant mortality rate at the egg stage was observed in the extracts of *P. odorata* and *L. aromatica* based on acetone and ethanol solvents. Other treatments influenced the development and growth of the larvae that hatch from a surviving egg, even though they have less of an effect on house fly egg mortality. According to the findings of the larvicidal bioassay, most plant extracts that employed hexane as a solvent caused a significant percentage of house fly larval deaths. Even when they are not immediately poisonous to insects, some plant extracts used in other treatments did have an impact on larval growth. The majority of plant extracts based on different solvents caused significant mortality (>80%) in house fly pupa. In this study, after applying certain plant extracts during the larval and pupal stages, the proportion of adult females was lower than that of males. Lethal concentration ($LC_{50,}$ LC_{90}) of high efficiency plant extract against immature stage of house flies were estimated for *L. aromatica*, *P. odorata*, *C. aurantium*, *M. paniculata*, *Co. esculenta*, *Ma. Esculenta* extracts. These extracts might have utility for controlling the immature stage of housefly in urban and veterinary areas.

Conflict of Interests

The author declares that they have no conflict of interests.

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