# Phytochemical-based Control and Larvicidal Impact of Hyptis suaveolens on Anopheles Mosquitoes

**Dr. Samson Raju. Cherukuri, Lecturer in Zoology, Government Degree College, Puttur, Tirupati District**

**Abstract:**

Preparing extracts with different solvents (such as water, ethanol, methanol, or chloroform) and testing them against mosquito larvae in a lab setting at varying concentrations and exposure times is the first step in evaluating the larvicidal potential of leaf extracts. The percentage of dead or moribund larvae is then recorded in order to calculate the lethal concentration (LC50 and LC90) values and overall efficacy. By determining which extracts and amounts efficiently kill mosquito larvae, this method finds strong, environmentally acceptable bio-larvicides for mosquito control. In this work, the larvicidal potential of ethanolic and aqueous leaf extracts of Hyptis suaveolens Poit was assessed on laboratory-reared Anopheles spp. larvae in their fourth larval instar at different doses of 0.1, 0.2, 0.3, 0.4, and 0.5 milliliters for 24 hours, 48 hours, and 72 hours. Bioactive components such as alkaloids, saponins, phenols, anthraquinones, and flavonoids were found by qualitative phytochemical screening of the leaf extracts. Significant larvicidal ability has been demonstrated by the leaf extracts of Hyptis suaveolens, or bush mint, against Anopheles mosquitoes, the main malaria vectors. Research shows that the plant's leaves contain bioactive substances that are poisonous to mosquito larvae in both ethanolic and aqueous preparations. Generally speaking, the extracts' efficacy is dose-dependent and rises with exposure time and concentration. It is therefore a viable and environmentally beneficial substitute for synthetic pesticides. The ethanolic leaf extracts of Hyptis suaveolens Poit exhibited the highest toxicity on the test organisms within 24 hours of exposure, with a median LC50 value of 0.485 ml, in contrast to the aqueous extract's LC50 value of 0.625 ml, according to the LC50 and LC90 values obtained. According to the relative median potency estimations, the ethanolic Hyptis suaveolens Poit was 0.161 times more effective than the aqueous form on the test organism in a 24-hour period. Thus, the study's findings highlight Hyptis suaveolens Poit's effectiveness as a sustainable substitute for controlling Anopheles mosquitoes.

To identify and purify toxic phytochemicals having larvicidal potential, it is thus advised that the extracts undergo quantitative phytochemical screening, column chromatography, and thin layer chromatography. Researchers use a conventional approach that involves gathering plant

material, making extracts using different solvents, performing bioassays on mosquito larvae, and assessing the outcomes in order to assess the larvicidal ability of leaf extracts. Finding and measuring the effectiveness of botanical chemicals as a sustainable substitute for synthetic pesticides is the aim.

**Keywords:** vector control, phytochemicals, fatal concentration, larvicidal capability, Anopheles species, and potency ratio. Anopheles mosquitoes, Hyptis suaveolens, plant extracts, larvicidal action, mosquito control, and malaria vector.

**1. Introduction:**

Malaria and other mosquito-borne illnesses continue to pose a serious threat to global public health. Although synthetic pesticides have been used for a long time to control mosquitoes, their excessive usage has resulted in the development of resistance, polluting of the environment, and adverse effects on creatures that are not the intended target. Given their biodegradability and environmental friendliness, botanicals provide interesting alternatives in this regard. The larvicidal activity of Hyptis suaveolens Poit leaf extracts against Anopheles mosquito larvae is assessed in this study. Mortality rates were noted following 24 and 48 hours of exposure to various solvent extracts (aqueous, methanolic, and ethanolic) at different concentrations. One of the worst illnesses in the world, malaria is most prevalent in tropical and subtropical areas where female Anopheles mosquitoes, the disease's vector, breed in large numbers.

In 2016, 91 countries and territories had continuous malaria transmission, with over half of the world's population—including parts of Africa, South-East Asia, the Eastern Mediterranean, the Western Pacific, and the Americas—at risk of contracting the disease [1]. A disproportionately large majority of the world's malaria cases occur in Africa. Ninety percent of malaria cases and ninety-one percent of malaria fatalities occurred in the region in 2016 [2]. In Africa, there were an estimated 216 million cases of malaria and 445,000 fatalities, the majority of which were children [3].

**Plant-specific efficacy:** The larvicidal potency of various plant species, as well as even distinct plant sections, might vary. For instance, research indicates that Dracaena loureiri's leaf extract works well against Aedes aegypti, but not its stem extract.

**Solvent-dependent potential:** The toxicity of the leaf extract is greatly influenced by the extraction solvent. The best solvents differ depending on the plant type and target insect, according to research findings.

In several nations, mosquitoes have become resistant to manmade pesticides in recent years [4]. This puts vulnerable populations at serious danger and jeopardizes malaria control efforts.

[1]. The focus on mosquito control has gradually changed from the use of traditional synthetic insecticides to alternative phytochemical products that are target-specific, biodegradable, economical, effective, and environmentally safe due to worries about the quality and safety of life as well as the environment. The effectiveness of extracts of several plant materials as potential substitutes in vector control programs has been reported in a number of studies [5,6,7]. In Gwandu Emirate (Aliero, Bagudo, Birnin-Kebbi, Bunza, Gwandu, Jega, Kalgo, Koko/Besse, Maiyama, and Suru LGAs) in Kebbi State, rural residents typically utilize the leaves of Hyptis suaveolens Poit, also known in Hausa as "Saurakwon sauro," to ward off mosquitoes.
Concentration-dependent mortality: Studies show a significant relationship between larval mortality and extract concentration. In general, a higher concentration leads to a higher death rate. Eco-friendly substitute: Research shows that botanical larvicides present a viable substitute for chemical pesticides, which are prone to resistance and environmental damage. Natural extracts are safe for non-target species, environmentally benign, and biodegradable. This suggests that it most likely contains bioactive substances that are harmful to mosquitoes. Therefore, the purpose of this article is to evaluate and contrast the effects of Hyptis suaveolens Poit leaf extracts in ethanol and water on Anopheles spp. larvae.
Hyptis suaveolens's rich phytochemical makeup is thought to be responsible for its larvicidal effectiveness. Alkaloids, saponins, flavonoids, phenols, and anthraquinones are among the bioactive substances found in the leaf extracts after qualitative screening. It is believed that these substances impair the mosquito larvae's physiological processes, resulting in their death. H. suaveolens is a good option for the development of botanical-based larvicides since the extracts include these compounds.

## 2. Methodology for evaluation

### 2.1. Preparation of leaf extracts

• **Gathering:** The target plant's leaves are gathered, cleaned, let to dry in the shade, and then crushed into a fine powder.

• **Extraction:** To separate the phytochemicals, the powdered leaves are exposed to various solvents, including water, ethanol, hexane, chloroform, or ethyl acetate. Maceration is a popular method that involves soaking the powdered material in the solvent for a while and then filtering it.

• **Concentration:** To leave a crude extract for testing, the filtered extract is concentrated using a vacuum evaporator to eliminate the solvent.

**2.2. Larvicidal bioassay:**

• **Larval collection:** Aedes aegypti, Anopheles stephensi, or Culex quinquefasciatus larvae of a target species are gathered or raised in a lab.

• **Test setup:** Different concentrations of the produced leaf extracts are given to many groups of larvae.

• **Control groups:** In addition to the test groups, a negative control consisting just of water (and occasionally the extract's solvent) is kept. For contrast, a recognized pesticide may also be employed as a positive control.

• **Observation:** Following a predetermined exposure time, usually 24, 48, or 72 hours, the number of dead and moribund larvae is noted.

**2.2.1. Collection and Preparation of Plant Materials**

Using sterile specimen bags, Hyptis suaveolens Poit plant leaves were gathered from the bushes in the Birnin-Kebbi region of Kebbi State. A plant taxonomist at Kebbi State University of Science and Technology, Aliero's Botany Unit of the Department of Biological Sciences, identified the leaves using voucher number 289A. The leaves were then laid out on a sanitized surface and given ample time to air-dry at room temperature in the shade after being cleaned with distilled water to get rid of any remaining dirt.

**2.3 Extraction of Plant Materials**

The maceration extraction procedure, as outlined by Odebiyi and Sofowora [9], was used to the dried leaves. An electric blender was used to grind them up. 500 milliliters of each solvent (ethanol and water) were used to soak fifty grams (50g) of each powdered leaf sample. The contents of a 1000 ml beaker were sealed with aluminum foil and stored in a cold, dry location. To prevent the growth of fungi, the contents were periodically agitated. The deadlines for the

ethanolic and aqueous extracts were 72 and 24 hours, respectively. Whatman filter paper was used in each instance, and the filtrates were then allowed to evaporate in a water bath that was set at 80°C until the solvents had gone.

Weighing and recording were done on the obtained crude ethanolic and aqueous extracts. To create the stock solution, roughly 1000 mg (1g) of the crude extract was redissolved in 100 cm of the suitable solvents (ethanol and water) in accordance with WHO standards [10]. Until the preparation of the diluted concentration for the larvicidal test, they were kept in the refrigerator.

**2.4. Screening for Qualitative Phytochemicals :**

Using the techniques outlined by [11,12], crude ethanolic and aqueous extracts of Hyptis spp. leaves were tested for phytochemicals.

**2.5 Anopheles spp. Rearing:**

In the Gesse Phase 1 region of Birnin-Kebbi, freshwater bodies such as gutters were used to gather Anopheles spp. larvae. Because their bodies were parallel to the water's surface, the mosquito larvae were recognized as Anopheles spp. [13]. The test organisms were raised in compliance with WHO recommendations [9]. They were moved to a clear plastic container within a net-lined cage that was around 26 x 10 x 6 cm in size. They were kept in a typical photoperiod and at ambient temperature.

Until the pupae appeared, the larvae were given a diet consisting of yeast. They were given a glucose meal (sugar solution) once they emerged as adults. The females were fed blood from pigeons that had been restrained and stripped of their feathers. In the larval container, eggs were often deposited at night. The eggs were gathered in the morning, placed in a tissue paper-lined beaker, and left at room temperature to allow the eggs to congregate and provide space for a uniform hatch. Once there were enough eggs, they were placed in a clear plastic container and submerged in distilled water. A little amount of larval food (yeast) was introduced to the water 24 hours prior to the addition of the eggs in order to encourage hatching.

Egg-hatching would occur as a result of the water being less oxygenated due to the bacterial development. Within 12 hours of hydration, this mechanism often caused the first instars to hatch [10]. Yeast was utilized as larval feeding. In order to prevent turbidity and scum, as well as significant bacterial growth that kills the larvae, the amount of yeast was maintained low. For around five to six days, the larvae were monitored every day until the distinctive fourth instars appeared [10].

**2.6. Data analysis:**

• **Mortality calculation:** For every concentration, the percentage of larval mortality is determined. Abbott's formula is used to adjust the findings if the negative control contains mortality.

• **Probit analysis**: A statistical technique for calculating the Lethal Concentration values, probit analysis is used to examine the data. The most widely used measures are: LC50: The concentration needed to kill half of the larvae that are exposed. LC90: The concentration needed to eliminate 90% of the larvae that are exposed.

• **Phytochemical screening:** To pinpoint the precise bioactive substances—such as flavonoids, alkaloids, saponins, and tannins—that are in charge of the larvicidal action, qualitative and quantitative tests are conducted on the best extracts.

• **Morphological analysis:** Under a microscope, dead larvae are inspected for any damage or abnormalities brought on by the extracts, such as intestinal destruction.

**2.7. Larvicidal Bioassay:**

The WHO-adopted standard test procedures were followed while performing the larvicidal bioassay [10]. Five milliliters of each stock solution were dissolved in forty-five milliliters of water to create the stock's diluted concentration, which was ten times. Twenty (20) healthy fourth instar larvae of Anopheles spp. (which can be identified from other previous instars by having a broad and heavily sclerotized collar on the posterior border of the head) were used to test the various concentrations of 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml of the individual extracts of Hyptis spp. [13]. 100ml of water and 150ml plastic containers were used for the experiments.

During each experiment, three duplicates of each concentration were run concurrently with a control. One milliliter of ethanol in one hundred milliliters of water served as the positive control, while one hundred milliliters of untreated water served as the negative control. The apparatus for the experiment was kept at ambient temperature. For three days, the number of dead and moribund larvae was counted daily (at 24-hour intervals) to assess each extract's larvicidal activity. For each concentration, the mean of the moribund and dead larvae in the three replicates was calculated and shown as the % mortality. Larvae that did not move after being prodded with a sterile needle for 10 seconds were identified as dead or moribund and noted. According to WHO [10], the percentage mortality is as follows:

$$\text{Mortality (\%)} = \frac{\text{Number of dead/moribund larvae}}{\text{Number of larvae introduced}} \times \frac{100}{1}$$

A small fraction of the larvae (between 5% and 20%) in the control batches perished throughout the experiment, which was adjusted for using Abbott's formula [14].

$$P = \frac{Pt - Pc}{100 - Pc} \times 100 \text{ or } P = \frac{X - Y}{X} \times 100$$

where X is the percentage survival in the untreated control, Y is the percentage survival in the treated sample, P is the corrected mortality, Pt is the observed mortality in the test sample, and Pc is the mortality in the control.

**2.7. 1. Statistical Analysis:**

SPSS version 20 was used to apply Probit Analysis, as explained by Finney [15]. Probit analysis was used to calculate the extracts' relative potency ratio and fatal concentration values ($LC_{50}$ and $LC_{90}$) at 24, 48, and 72 hours. Estimates of the extracts' larvicidal efficacies were also made.

**2.8. Assessing Potential Larvicidal Effects:**

**1.Preparation of Plant Materials:**

Gather the target plant species' leaves and let them dry in the shade. Make a fine powder out of the dried leaves.

**2. Extract Preparation:**

Utilize a Soxhlet extractor to extract the plant material in powder form using various solvents, including ethanol, water, petroleum ether, methanol, and chloroform. The target molecules determine the solvent selection, which has a big impact on effectiveness.

**3. Larval Bioassays:** In a lab environment, expose early fourth-instar mosquito larvae to different doses of the produced leaf extracts. To check for natural mortality, include a control group that isn't given any extract.

**4. Data Collection:** Over the course of many days, count the dead and moribund (stuck and dying) larvae at predetermined intervals (e.g., 24 hours). Determine the death rate as a percentage for every concentration. To account for any mortality in the control group, use Abbott's formula.

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**5. Statistical Analysis:** Determine the Lethal Concentration 50 (LC50), which is the concentration needed to kill 50% of the larvae, and the LC90, which is the concentration needed to kill 90%, using statistical analysis techniques like probit analysis.

**3. Result:**

• **Potency:** A stronger extract with a higher larvicidal action is indicated by a lower LC50 and LC90 score. Its Eco-Friendly Potential and that is To manage disease-transmitting mosquitoes like Aedes aegypti, extracts with strong larvicidal activity may be created as eco-friendly bio-larvicides.

**• Identification of Bioactive components:**

The extracts' toxicity is influenced by the presence of bioactive components, including anthraquinones, alkaloids, phenols, saponins, and flavonoids.

**3.1. Leaf Extract Qualitative Phytochemical Screening:**

Bioactive substances including alkaloids, saponins, tannins, phenols, anthraquinones, flavonoids, and steroids were detected in the leaf sample extracts of Hyptis suaveolens Poit using qualitative phytochemical screening (Table 1).

**3.2. LC50 and LC90, the median lethal concentrations:**

The mortality of Anopheles spp. larvae exposed to different extract concentrations across a range of time periods was used to test for resistance. The ethanolic and aqueous extracts' respective LC50 values at 24 hours are 0.485 and 0.625 (Table 2).

**3.3 Larvicidal Potential of the Leaf Extracts:**

The fourth instar stage of Anopheles spp. was used to test the larvicidal ability of Hyptis spp. leaves. When tested against the test organism, every extract showed larvicidal ability. The outcome shows that a higher dose or concentration causes a higher death rate. Ethanolic extract exhibited a total mortality of 88% at the maximum dose of 0.5 ml, whereas aqueous extract had a total mortality of 54.5% (Table 3). The test organism exhibited the maximum mortality rate of 55% within the first 24 hours of exposure to the extract at the highest concentration of 0.5 ml of the ethanolic extract, according to the lethality pattern of the ethanolic leaf extract of Hyptis suaveolens Poit on Anopheles spp. Mortalities over the 48- and 72-hour periods were between 10 and 20 percent. (Figs. 1, 2 and 3).

### 3.4 Relative Potency Ratio:

Potency or the reciprocal of an equitoxic dosage or concentration is used to compare the toxicities of two or more larvicides against an organism. Probit Analysis was used to compare the extracts' relative potencies. According to the results, the ethanolic extract outperformed the aqueous extract by 0.161 times at 24 hours, 0.49 times at 48 hours, and 0.4 times at 72 hours (Table 4).

Table1: Results of Phytochemical Screening of Hyptis suaveolens Poit Leaf Sample Extracts

| Secondary metabolites | Hexane | Trichloromethane | Ethylacetate | Ethanol | Water |
|---|---|---|---|---|---|
| Alkaloids | – | – | + | – | – |
| Coumarins | – | – | + | + | – |
| Flavonoids | – | – | – | – | + |
| Phenylpropanoids | – | – | – | + | – |
| Saponins | – | – | – | – | + |
| Tannins | – | – | – | + | + |
| Terpenoids | + | + | – | – | – |

| Phytochemical | *Hyptis suaveolens* Poit | |
|---|---|---|
| | Ethanolic | Aqueous |
| Alkaloid | + | + |
| Saponin | + | + |
| Phenol | + | + |
| Tannin | + | + |
| Anthraquinone | + | + |
| Glycoside | – | – |
| Flavonoid | + | + |
| Steroid | – | + |
| Terpenoid | – | – |

Table 2: Hyptis spp. extracts' LC50 and LC90 values on Anopheles spp. at different times at a 95% confidence level (C. L.)

| Extract | Relative Median Potency at 95% C.L | | | LOG Transform | | |
|---|---|---|---|---|---|---|
| | Potency | Lower bound | Upper bound | Log potency | Lower bound | Upper bound |
| 24hrs HE | 3.359 | 1.659 | 5.059 | 0.526 | 0.026 | 1.026 |
| HA | 3.198 | 1.035 | 4.605 | 0.504 | 0.004 | 1.004 |
| 48hrs HE | 1.835 | 1.135 | 3.535 | 0.263 | -0.237 | 0.763 |
| HA | 1.345 | -0.355 | 3.045 | 0.128 | -0.372 | 0.628 |
| 72hrs HE | 1.756 | 0.056 | 3.456 | 0.756 | 0.256 | 1.256 |
| HA | 1.356 | -0.344 | 3.056 | 0.132 | -0.365 | 0.635 |

| Time (hrs) | Extract | Lethality | Conc (ml) | Lower bound | Upper bound | Log conc | Lower bound | Upper bound |
|---|---|---|---|---|---|---|---|---|
| 24 | ET | $LC_{50}$ | 0.485 | -0.215 | 1.185 | -0.314 | -0.414 | -0.214 |
| | | $LC_{90}$ | 0.970 | 0.270 | 1.670 | -0.013 | -0.113 | 0.087 |
| | AQ | $LC_{50}$ | 0.625 | -0.075 | 1.325 | -0.204 | -0.304 | -0.104 |
| | | $LC_{90}$ | 1.250 | 0.550 | 1.950 | 0.096 | -0.004 | 0.196 |
| 48 | ET | $LC_{50}$ | 1.388 | 0.688 | 2.088 | 0.142 | 0.042 | 0.242 |
| | | $LC_{90}$ | 2.777 | 2.077 | 3.477 | 0.443 | 0.343 | 0.543 |
| | AQ | $LC_{50}$ | 0.765 | 0.065 | 1.465 | -0.116 | -0.216 | -0.016 |
| | | $LC_{90}$ | 1.534 | 0.834 | 2.234 | 0.185 | 0.085 | 0.285 |
| 72 | ET | $LC_{50}$ | 1.000 | 0.300 | 1.700 | 0.000 | -0.100 | 0.100 |
| | | $LC_{90}$ | 2.000 | 1.300 | 2.700 | 0.301 | 0.201 | 0.401 |
| | AQ | $LC_{50}$ | 1.923 | 1.223 | 2.623 | 0.283 | 0.183 | 0.383 |
| | | $LC_{90}$ | 3.846 | 3.146 | 4.546 | 0.585 | 0.485 | 0.685 |

### 4. Results:

- **Dose-dependent mortality:** Larval mortality rose as extract concentration and exposure time increased.
- **Effect of the solvent:** In general, ethanolic and methanolic extracts exhibited more activity than aqueous extracts.

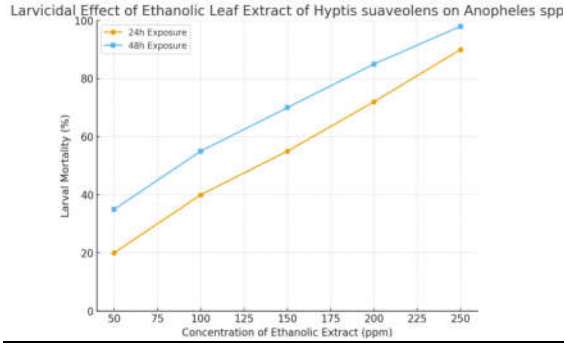
• **Efficacy:** It was discovered that $LC_{50}$ values were within the effective larvicidal range, which in certain cases was less than 200 ppm.

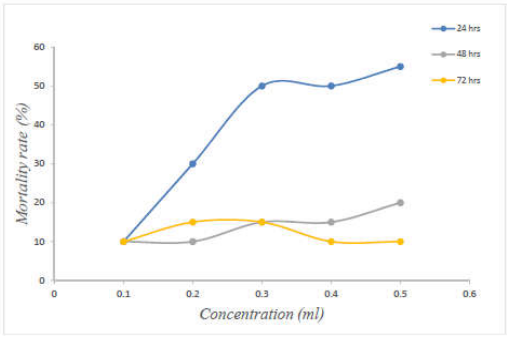
The following bioactive substances were found in this study using qualitative phytochemical screening: alkaloids, saponins, flavonoids, tannins, phenols, anthraquinones, and steroids (Table 1). This is consistent with research by Ohimain et al. [5], who found that these phytochemicals were present in Hyptis spp. that were highly toxic to Anopheles gambiae larvae, and Sakthivadivel et al. [16], who also found that these phytochemicals were present in Hyptis suaveolens Poit extracts that had high

| Concentration (ml) | Mortality (%) | | | Total |
|---|---|---|---|---|
| | Ethanolic | | | |
| | 24hrs | 48hrs | 72hrs | |
| Control | 0 | 2.5 | 0 | 2.5 |
| 0.1 | 10.00 | 11.65 | 11.65 | 33.30 |
| 0.2 | 28.30 | 11.65 | 13.30 | 53.25 |
| 0.3 | 51.50 | 13.30 | 13.30 | 78.10 |
| 0.4 | 50.00 | 16.50 | 10.00 | 76.50 |
| 0.5 | 55.00 | 21.50 | 11.50 | 88.00 |
| | | Aqueous | | |
| Control | 5 | 0 | 0 | 5 |
| 0.1 | 6.50 | 6.50 | 6.50 | 19.50 |
| 0.2 | 15.00 | 11.50 | 6.50 | 33.00 |
| 0.3 | 30.00 | 10.00 | 5.00 | 45.00 |
| 0.4 | 35.00 | 10.00 | 10.00 | 55.00 |
| 0.5 | 46.50 | 5.00 | 3.00 | 54.50 |

| Concentration (ml) | Mortality (%) | | | |
|---|---|---|---|---|
| | Ethanolic | | | |
| | 24hrs | 48hrs | 72hrs | Total |
| Control | 5 | 0 | 0 | 5 |
| 0.1 | 8.00 | 15.00 | 8.00 | 31.00 |
| 0.2 | 20.00 | 8.00 | 16.50 | 44.50 |
| 0.3 | 36.50 | 8.00 | 5.00 | 49.50 |
| 0.4 | 45.00 | 6.50 | 16.50 | 68.00 |
| 0.5 | 51.50 | 18.00 | 25.00 | 94.50 |
| | | Aqueous | | |
| Control | 0 | 0 | 0 | 0 |
| 0.1 | 3.00 | 6.50 | 8.00 | 17.50 |
| 0.2 | 11.50 | 1.50 | 8.00 | 21.00 |
| 0.3 | 21.50 | 8.00 | 5.00 | 34.50 |
| 0.4 | 26.50 | 6.50 | 6.50 | 39.50 |
| 0.5 | 40.00 | 10.00 | 13.00 | 63.00 |

Table 3: Hyptis suaveolens poit leaf extracts' larvicidal efficacy on Anopheles species




Alkaloids, saponins, tannins, anthraquinones, steroids, and other phytochemicals have been demonstrated to have larvicidal effects on mosquitoes, according to et al. [17]. It has been observed that the phytochemicals found in the leaves of the Lamiaceae family, which includes Hyptis suaveolens Poit, have larvicidal effects on mosquitoes [5]. This supports the finding that some phytochemicals, including tannin, alkaloids, flavonoids, and saponins, have larvicidal properties against mosquitoes [18,19,20]. Although they displayed varying degrees of toxicity to the test organism, the ethanolic and aqueous leaf extracts of Hyptis suaveolens Poit employed in this investigation nearly contained the same classes of phytochemicals.

The antagonistic impact of any interaction between two or more phytochemicals, as well as variations in the concentration of each phytochemical in each leaf extract, might be the cause of this. Alkaloids and saponins have been shown to have an antagonistic influence on quinine tree bioactivity [21]. The study's findings demonstrated a significant relationship between the extract content and the larvae's death rate. A close examination of the relationship between concentration and mortality rate makes this clear: higher concentrations result in higher mortalities. This is consistent with the findings of Babatunde et al. [22], who found that greater mortality was a result of higher extract concentrations.

In the bioassay for both Anopheles spp and Culex spp of this study, it can be observed that the highest mortalities were achieved within the first 24hrs of exposure of the test organisms to the extracts. This disagrees with the results obtained by Mahmoud et al. [23] and Sakthivadivel et al. [16] which reported highest mortality at 72hrs.

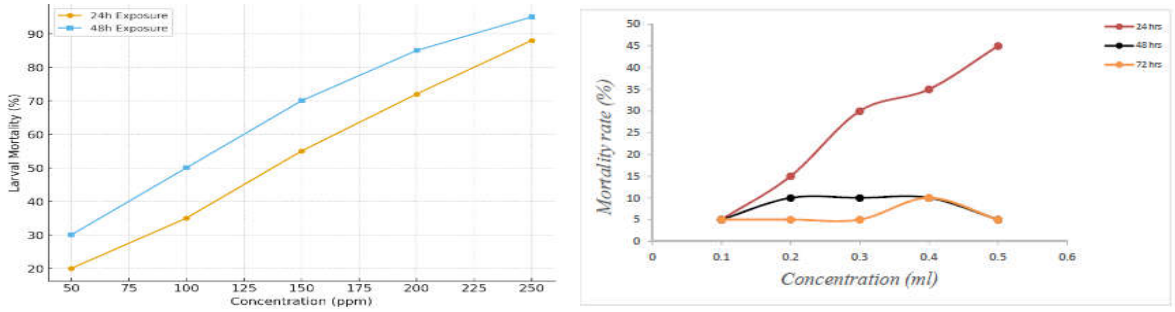



**Fig. 2.** Hyptis spp. aqueous leaf extract's larvicidal efficacy on Anopheles spp.

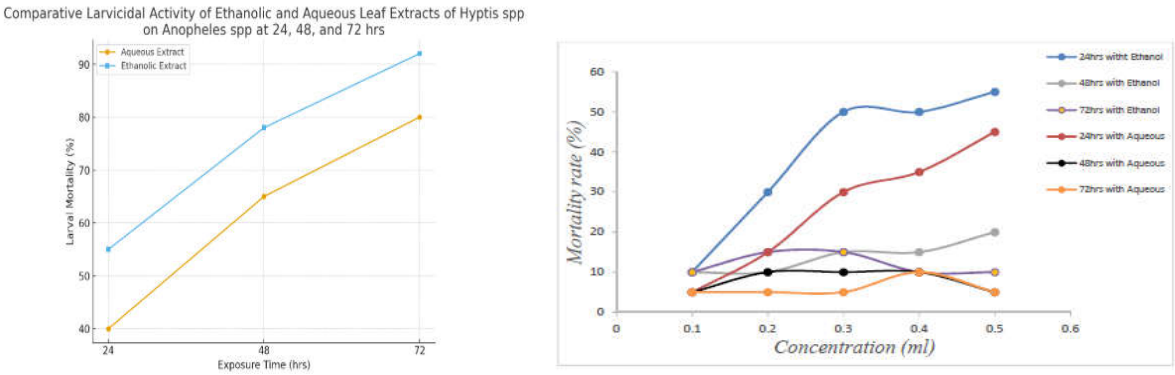



Fig.3. Comparison of the larvicidal effects of ethanolic and aqueous Hyptis spp. leaf extracts on Anopheles spp. at 24, 48, and 72 hours

| Extract Type | $LC_{50}$ (ppm) | Reference Extract (Aqueous $LC_{50}$ = 180 ppm) | RMPR | Relative Potency |
|---|---|---|---|---|
| Aqueous | 180 | 180/180 | 1.00 | Baseline |
| Ethanolic | 120 | 180/120 | 1.50 | 1.5× more potent |
| Methanolic | 100 | 180/100 | 1.80 | 1.8× more potent |
| Hexane | 220 | 180/220 | 0.82 | Less potent |

| Extract | | Relative Median Potency at 95% C.L | | | LOG Transform | | |
|---|---|---|---|---|---|---|---|
| | | Potency | Lower bound | Upper bound | Log potency | Lower bound | Upper bound |
| 24hrs | HE | 3.359 | 1.659 | 5.059 | 0.526 | 0.026 | 1.026 |
| | HA | 3.198 | 1.035 | 4.605 | 0.504 | 0.004 | 1.004 |
| 48hrs | HE | 1.835 | 1.135 | 3.535 | 0.263 | -0.237 | 0.763 |
| | HA | 1.345 | -0.355 | 3.045 | 0.128 | -0.372 | 0.628 |
| 72hrs | HE | 1.756 | 0.056 | 3.456 | 0.756 | 0.256 | 1.256 |
| | HA | 1.356 | -0.344 | 3.056 | 0.132 | -0.365 | 0.635 |

*Key: HE – Hyptis Ethanolic, HA – Hyptis Aqueous*

Table 4. Relative Median Potency Ratio of *Hyptis suaveolens* Leaf Extracts against *Anopheles* spp

To prepare a **table on Relative Median Potency Ratio (RMPR)**, we use the **$LC_{50}$ (lethal concentration that kills 50% of larvae)** values of the different extracts and compare them.

The **Relative Median Potency Ratio (RMPR)** is usually calculated as:

- $$\text{RMPR} = \frac{LC_{50} \text{ of reference extract}}{LC_{50} \text{ of test extract}}$$
- If RMPR > 1 → Test extract is **more potent** than the reference.
- If RMPR < 1 → Test extract is **less potent** than the reference.

The phytochemicals found in H. suaveolens, including terpenoids, alkaloids, flavonoids, and essential oils, may be responsible for the larvicidal action. Plant-based extracts provide environmentally acceptable and biodegradable substitutes for synthetic larvicides. The findings corroborate past studies on H. suaveolens's insecticidal capabilities. Hyptis suaveolens Poit's ethanolic leaf extracts had LC50 and LC90 values of 0.485 and 0.970 milliliters, respectively, whilst its aqueous extracts have LC50 and LC90 values of 0.625 and 1.250 milliliters, respectively, throughout a 24-hour period (Table 2). This demonstrates unequivocally that the treatment employing ethanolic leaf extracts had the greatest test organism fatality rate.

Even though this study showed that an increase in the concentration of the leaf extracts caused Anopheles spp. mortality to rise in proportion, an increase in the test organism's exposure time to the leaf extracts did not always result in an increase in mortality. In the first 24 hours, the test organism was most mortalized by ethanolic and aqueous extracts, with ethanolic Hyptis species being the most effective. After 72 hours, the test organism had the maximum mortality rate of

88% from ethanolic Hyptis spp., closely followed by its aqueous extract, which had a mortality impact of 54.5%.

According to the study's relative potency ratio, ethanolic Hyptis suaveolens Poit leaf extract had 0.161 times the potency of aqueous Hyptis suaveolens Poit leaf extract on the test organism after 24 hours. This may be because organic phytochemicals have a propensity to dissolve more readily in ethanol than in water [10].

**1. Plant Collection and Preparation:** Fresh Hyptis suaveolens leaves were gathered, cleaned, shade-dried, and ground into powder. Soxhlet extraction or maceration were used to create extracts utilizing solvents (aqueous, ethanol, methanol, hexane, etc.).

**2. Larval Collection and Rearing:** Third and fourth instar Anopheles mosquito larvae were either raised in a controlled laboratory environment or obtained from environments with stagnant water.

**3. Bioassay:** Various plant extract concentrations (e.g., 50, 100, 150, 200, and 250 ppm) were made.

• Each concentration was administered in triplicate to groups of 25 larvae.

• Distilled water and solvent control groups were kept in place.

• Mortality was noted 24 and 48 hours later.

**4. Analysis of Data :**

• Abbott's formula was used to compute the percentage mortality.

• Probit analysis was used to determine the $LC_{50}$ and $LC_{90}$ values.

**5. Conclusion:**

According to research, Hyptis suaveolens' ethanolic extract is often more poisonous and powerful to Anopheles larvae than its aqueous extract. One study, for instance, discovered that following 24 hours of exposure, the ethanolic extract had a lower median lethal concentration (LC50) than the aqueous extract. Greater toxicity is indicated by a lower LC50 value, which means that less of the drug is required to kill 50% of the test organisms. The use of synthetic pesticides in vector control programs has led to a number of issues throughout the years, such as pesticide resistance, toxicity to humans and other non-target animals, and environmental contamination. Therefore, the evaluation and creation of substitute goods that are affordable, safe for the environment, biodegradable, and effective against these disease vectors are given a lot of importance. Humanity is endowed with powerful

phytochemicals that fulfill these needs, according to studies [7]. The diverse variety of active organic chemicals is frequently credited with the plant extract's effectiveness. Plant-derived insecticides are botanical mixtures of chemical compounds that work together to affect the physiological and behavioral processes of target species, in contrast to traditional insecticides that are based on a single active ingredient. As a result, the likelihood of pests becoming resistant to these compounds is quite low [7]. The study's findings have significant ramifications for the actual use of plant extracts in mosquito control. This is due to the fact that mosquitoes must develop as larvae in freshwater environments; therefore, it is more economical and advantageous to stop their development at this point rather than letting them mature into adults that might endanger human comfort and health. These plant extracts are affordable, simple to make, and harmless for the environment when used to control mosquitoes. They have the potential to be utilized directly as larvicidal agents in aquatic ecosystems with limited volumes in and near human habitations. In order to effectively control mosquitoes, it is hoped that the results of this study will spur additional research into the evaluation, identification, bioassay-guided fractionation, isolation, and purification of bioactive ingredients, particularly from the crude extracts of Hyptis suaveolens Poit leaves, and their systemic effects on target mosquitoes. This could allow for the standardization and practical application of the extract as a larvicide in small-volume aquatic habitats or breeding sites of limited size in and around human settlements. Since Hyptis suaveolens leaf extracts have been shown to be effective, they may be applied directly to mosquito breeding grounds, such as standing water bodies, to kill the larvae before they can develop into biting adults. This is known as larval source management.

The purpose of biopesticide development is to The development of a standardized, commercially accessible bio-pesticide that is safe for the environment and non-target species may result from additional study and purification of the extracts. In certain areas, the plant has long been used to control mosquitoes because to its well-known insect-repelling qualities. Hyptis suaveolens leaf extracts, particularly methanolic and ethanolic extracts, show strong larvicidal efficacy against Anopheles mosquito larvae. Following more field testing and toxicological research, the plant may be produced as an affordable, environmentally friendly larvicide.

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